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On the Origin of Mitosing Cells

LYNN SAGAN

Department of Biology, Boston University
Boston, Massachusetts, U.S.A.

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A theory of the origin of eukaryotic cells ("higher" cells which divide by classical mitosis) is presented. By hypothesis, three fundamental organelles: the mitochondria, the photosynthetic plastids and the (9+2) basal bodies of flagella were themselves once free-living (prokaryotic) cells. The evolution of photosynthesis under the anaerobic conditions of the early atmosphere to form anaerobic bacteria, photosynthetic bacteria and eventually blue-green algae (and protoplastids) is described. The subsequent evolution of aerobic metabolism in prokaryotes to form aerobic bacteria (protoflagella and protomitochondria) presumably occurred during the transition to the oxidizing atmosphere. Classical mitosis evolved in protozoan-type cells millions of years after the evolution of photosynthesis. A plausible scheme for the origin of classical mitosis in primitive amoeboflagellates is presented. During the course of the evolution of mitosis, photosynthetic plastids (themselves derived from prokaryotes) were symbiotically acquired by some of these protozoans to form the eukaryotic algae and the green plants.

The cytological, biochemical and paleontological evidence for this theory is presented, along with suggestions for further possible experimental verification. The implications of this scheme for the systematics of the lower organisms is discussed.

1. Introduction

All free-living organisms are cells or are made of cells. There are two basic cell types: *prokaryotic* and *eukaryotic*. Prokaryotic cells include the eubacteria, the blue-green algae, the gliding bacteria, the budding bacteria, the pleuropneumonia-like organisms, the spirochaetes and rickettsias, etc. Eukaryotic cells, of course, are the familiar components of plants and animals, molds and protozoans, and all other "higher" organisms. They contain subcellular organelles such as mitochondria and membrane-bounded nuclei and have many other features in common.

> "The numerous and fundamental differences between the eukaryotic and prokaryotic cell which have been described in this chapter have been fully recognized only in the past few years. In fact, this basic divergence in cellular structure which separates the bacteria and blue-green algae

from all other cellular organisms, probably represents the greatest single evolutionary discontinuity to be found in the present-day living world" (Stanier, Douderoff & Adelberg, 1963).

This paper presents a theory of the origin of this discontinuity between eukaryotic (mitosing or "higher") and prokaryotic cells. Specifically, the mitochondria, the (9+2) basal bodies of the flagella, and the photosynthetic plastids can all be considered to have derived from free-living cells, and the eukaryotic cell is the result of the evolution of ancient symbioses. Although these ideas are not new [Merechowsky (1910) & Minchin (1915) in Wilson (1925), Wallin (1927), Lederberg (1952), Haldane (1954), Ris & Plaut (1962)],† in this paper they have been synthesized in such a way as to be consistent with recent data on the biochemistry and cytology of subcellular organelles. In accord with both the fossil record and this theory, the lower eukaryotes (protozoans, eukaryotic algae and fungi) can now be included on a single phylogenetic tree (Fig. 1). In contrast to previous thought on the subject (Cronquist, 1960; Dougherty & Allen, 1960; Fritsch, 1935), many aspects of this theory are verifiable by modern techniques of molecular biology.

In defending the idea that the eukaryotic cell arose by a specific series of endosymbioses, a plausible scheme for the origin of mitosis itself emerges. ("Mitosis" is meant only in the classical sense; the analogous equal distribution of genes to daughter cells in prokaryotes is not relevant here.)

The paper is organized into three parts. The first presents the theory of the origin of the eukaryotic cell. The second part is a compilation of the scientific literature in support of the sequences presented first. The last section suggests some experimental results which can be predicted from the theory.

2. Hypothetical Origin of Eukaryotic Cells

2.1. THE EVOLUTION OF PROKARYOTIC CELLS IN THE REDUCING ATMOSPHERE

Prokaryotic cells containing DNA, synthesizing protein on ribosomes, and using messenger RNA as intermediate between DNA and protein, are ancestral to all extant cellular life. Such cells arose under reducing conditions of the primitive terrestrial atmosphere ($4{\cdot}5$–$2{\cdot}7 \times 10^9$ years ago). All earlier events leading to the origin of a population of free-living entities upon which natural selection could act (i.e. cells) are outside the province of this

† "More recently, Wallin (1922) has maintained that chondriosomes may be regarded as symbiotic bacteria whose associations with other cytoplasmic components may have arisen in the earliest stages of evolution ... to many, no doubt, such speculations may appear too fantastic for present mention in polite biological society; nevertheless, it is in the range of possibility that they may some day call for more serious consideration" (Wilson, 1925, see p. 378).

discussion, in other words they occurred prior to the events described herein (Bernal, 1957).

2.2. THE EVOLUTION OF PORPHYRIN SYNTHETIC PATHWAYS, PHOTOSYNTHESIS, AND RESPIRATION IN PROKARYOTIC CELLS

Early in the history of prokaryotic cells, nucleotide sequences (genes) which coded for porphyrin syntheses, evolved. The following is considered to be a plausible historical sequence: photodisassociation of water vapor in the upper atmosphere resulted in the escape of free hydrogen which led to the production of molecular oxygen. This threatened the highly reduced nucleic acid of early self-replicating cellular systems. Associated metal-chelated porphyrins protected these systems from oxidation. Genes coding for the pathways involved in the syntheses of such chelated porphyrins (e.g. the coenzymes of peroxidase and catalase) were selected for and retained under this continuing threat of naturally produced oxidizing agents. The incidental fact that such antimutagenic compounds preferentially absorb visible light was put to evolutionary advantage: cells eventually evolved containing mechanisms to produce ATP (and the other nucleotides) utilizing visible solar energy absorbed by chlorophyll-like porphyrins. These cells, now photosynthetic, replaced earlier ones in which ATP was synthesized by direct ultraviolet absorption. With energy released from photoproduced ATP, H atoms from atmospheric hydrogen and hydrogen sulfide were used in the reduction of CO_2 for cell material.

In other populations of heterotrophic microbes dependent upon fermentation of sugars and amino acids for ATP production, cells which used their porphyrins for more efficient oxidation of carbohydrates were eventually selected for. This resulted in microbes capable of cytochrome-mediated production of ATP *via* electron transport systems (e.g. anaerobic respirers: nitrate and sulfate reducers, etc.).

Eventually, among the primitive phototrophs, a population of cells arose using photoproduced ATP with water as the source of hydrogen atoms in the reduction of CO_2 for the production of cell material. This led to the formation of gaseous oxygen as a by-product of photosynthesis. Such oxygen elimination by microbial photosynthesizers increased the partial pressure of oxygen in the atmosphere. In the anaerobic respirers, the abundance, at least in some locations, of free oxygen led to the evolution of the final and aerobic step in respiration, i.e. the complete oxidation of carbohydrate *via* the cytochrome system with the elimination of CO_2 and H_2O.

Among autotrophic microbes which used porphyrins in anaerobic photosynthesis in the light, mutants which could use these same porphyrins for aerobic respiration in the absence of light were eventually selected for. This

resulted in the evolution of prokaryotic algae ancestral to extant blue-greens —with both photosynthetic and respiratory mechanisms for ATP production. These versatile prokaryotes represented a large step in cellular efficiency. They continued to eliminate gaseous oxygen in photosynthesis and further accelerated the transition to the oxidizing atmosphere. Thus, some time after the origin of the earth and before the deposition of oxidized rocks and micro-fossils—a period of about 2400 million years—populations of prokaryotes with the major photosynthetic and respiratory metabolic capabilities evolved ($4{\cdot}5$–$2{\cdot}1 \times 10^9$ years ago).

2.3. THE EVOLUTION OF EUKARYOTIC CELLS FROM PROKARYOTIC CELLS BY SYMBIOSIS

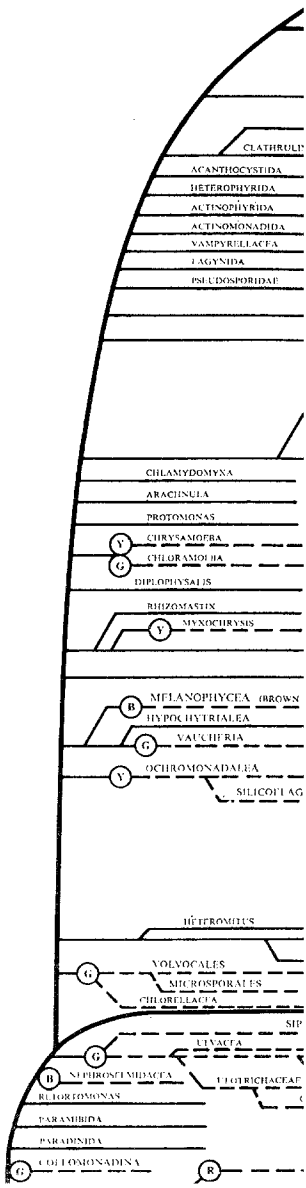
The continued production of free oxygen as a by-product of photosynthesis resulted in a crisis: all cells had to adapt to an atmosphere containing oxygen, or they had to survive in a specialized anaerobic environment. The geological evidence indicates oxygen was present in the atmosphere as early as $2{\cdot}7 \times 10^9$ years ago and became relatively abundant $1{\cdot}2 \times 10^9$ years ago (Cloud, 1965). By this time, then, all production of abiogenic organic matter must have come to a halt; not only was its source of energy removed by ozone absorption of ultraviolet light in the upper atmosphere, but such organic matter, if it were produced, would have been rapidly oxidized (Abelson, 1963). Therefore all terrestrial life became dependent, either directly or indirectly, upon cellular photosynthesis before $1{\cdot}2 \times 10^9$ years ago. To insure replication of their nucleic acids, heterotrophs were forced to eat organic matter produced by photosynthetic or chemoautotrophic processes.

It is suggested that the first step in the origin of eukaryotes from prokaryotes was related to survival in the new oxygen-containing atmosphere: an aerobic prokaryotic microbe (i.e. the protomitochondrion) was ingested into the cytoplasm of a heterotrophic anaerobe. This endosymbiosis became obligate and resulted in the evolution of the first aerobic amitotic amoeboid organisms.

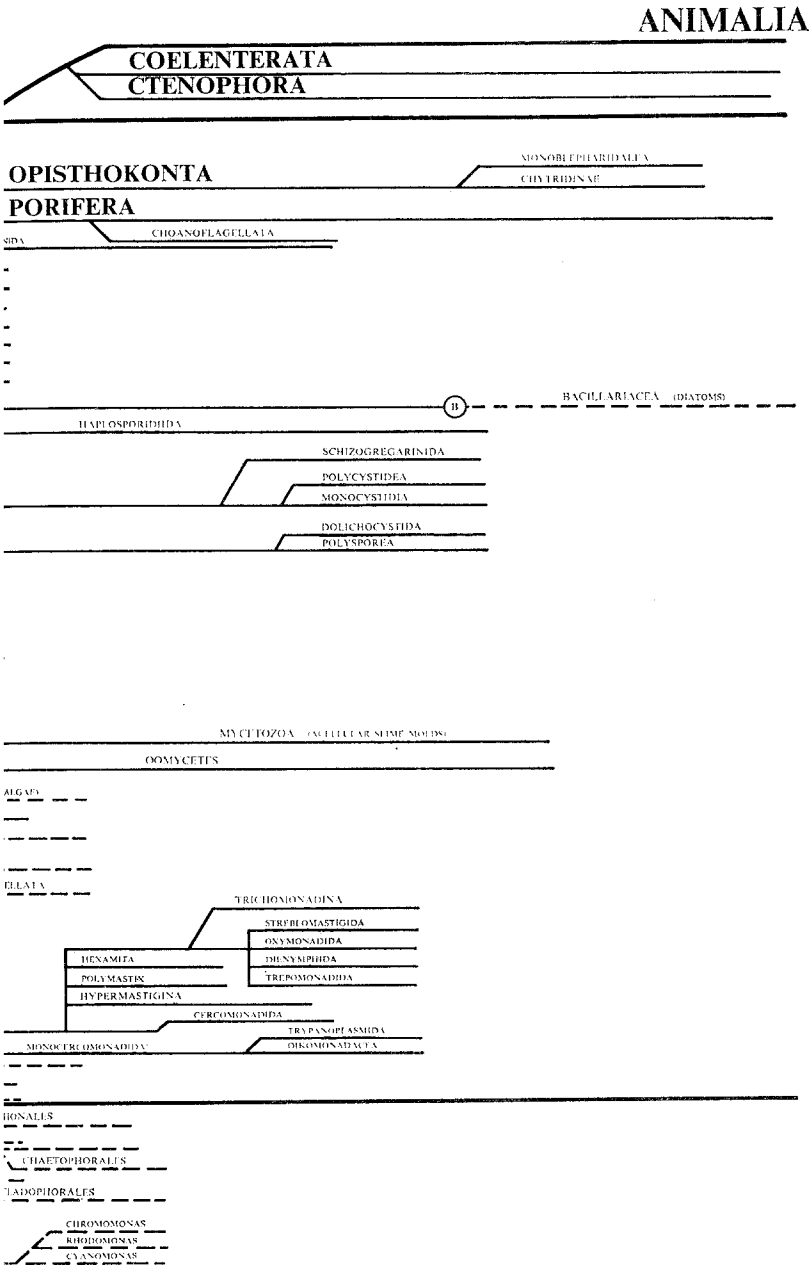
By hypothesis, some of these amoeboids ingested certain motile prokaryotes. Eventually these, too, became symbiotic in their hosts. The association of the motile prokaryote with the amoeboid formed primitive amoeboflagellates. In these heterotrophic amoeboflagellates classical mitosis evolved.

The evolution of mitosis, insuring an even distribution of large amounts of nucleic acid (i.e. host chromosomes containing host genes) at each cell division, must have taken millions of years. It most likely occurred after the transition to the oxidizing atmosphere, since all eukaryotic organisms

VI
(9 + 2) Homologue used both as basal body to flagella and other (9 + 2) homologue permanently differentiated as extranuclear division centers (centrioles).



V
(9 + 2) Homologue used both as basal body to flagella and as extranuclear division center.


ANIMALIA
COELENTERATA
CTENOPHORA
OPISTHOKONTA
MONOBLEPHARIDALEA
CHYTRIDINAE
PORIFERA
CHOANOFLAGELLATA
BACILLARIACEA (DIATOMS)
HAPLOSPORIDIDA
SCHIZOGREGARINIDA
POLYCYSTIDEA
MONOCYSTIDEA
DOLICHOCYSTIDA
POLYSPOREA
MYCETOZOA (CELLULAR SLIME MOULDS)
OOMYCETES
TRICHOMONADINA
STREBLOMASTIGIDA
OXYMONADIDA
HEXAMITA
DIPLOMONADIDA
POLYMASTIX
TREPOMONADIDA
HYPERMASTIGINA
CERCOMONADIDA
TRYPANOPLASMIDA
MONOCERCOMONADIDAE
DIKOMONADACEA
CHAETOPHORALES
CHROMOMONAS
RHODOMONAS
CYANOMONAS

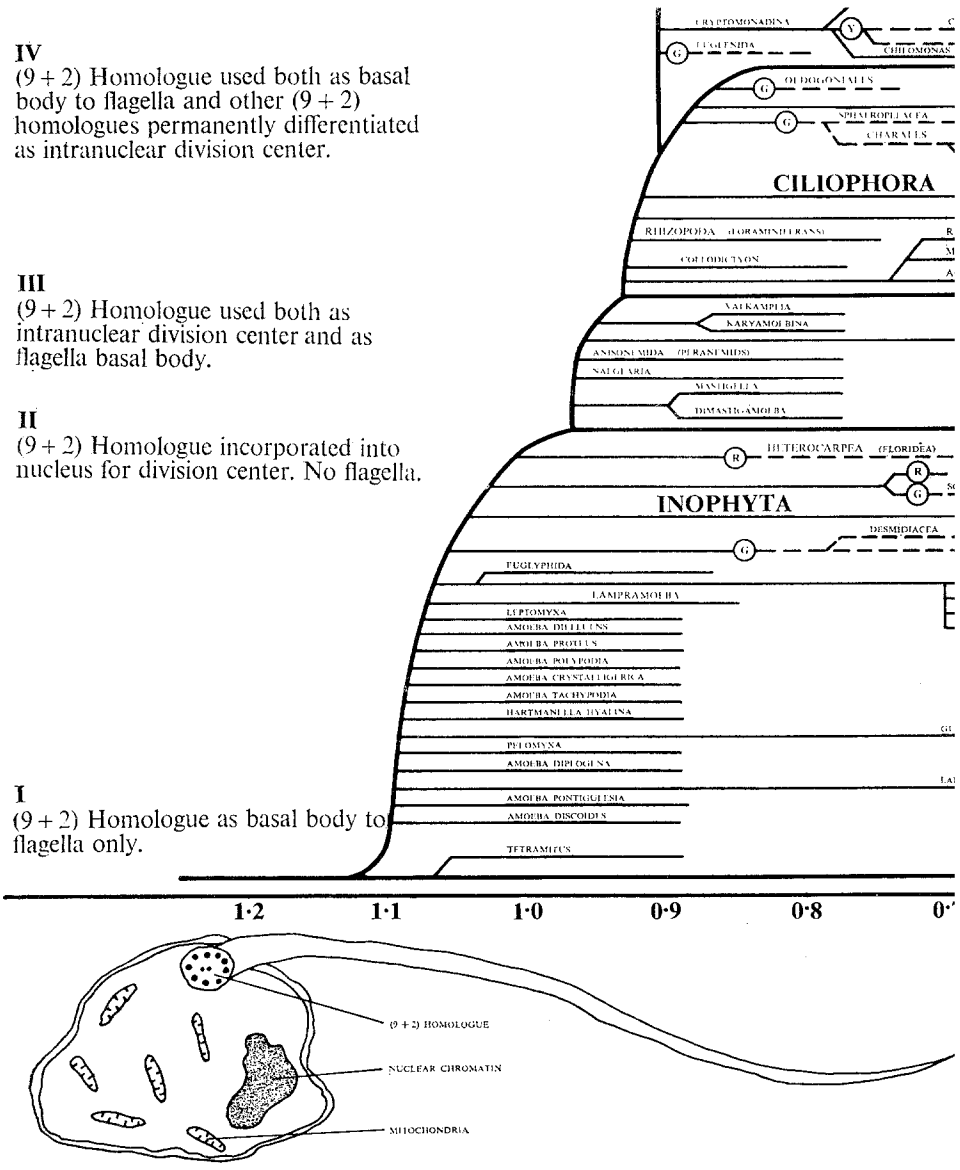
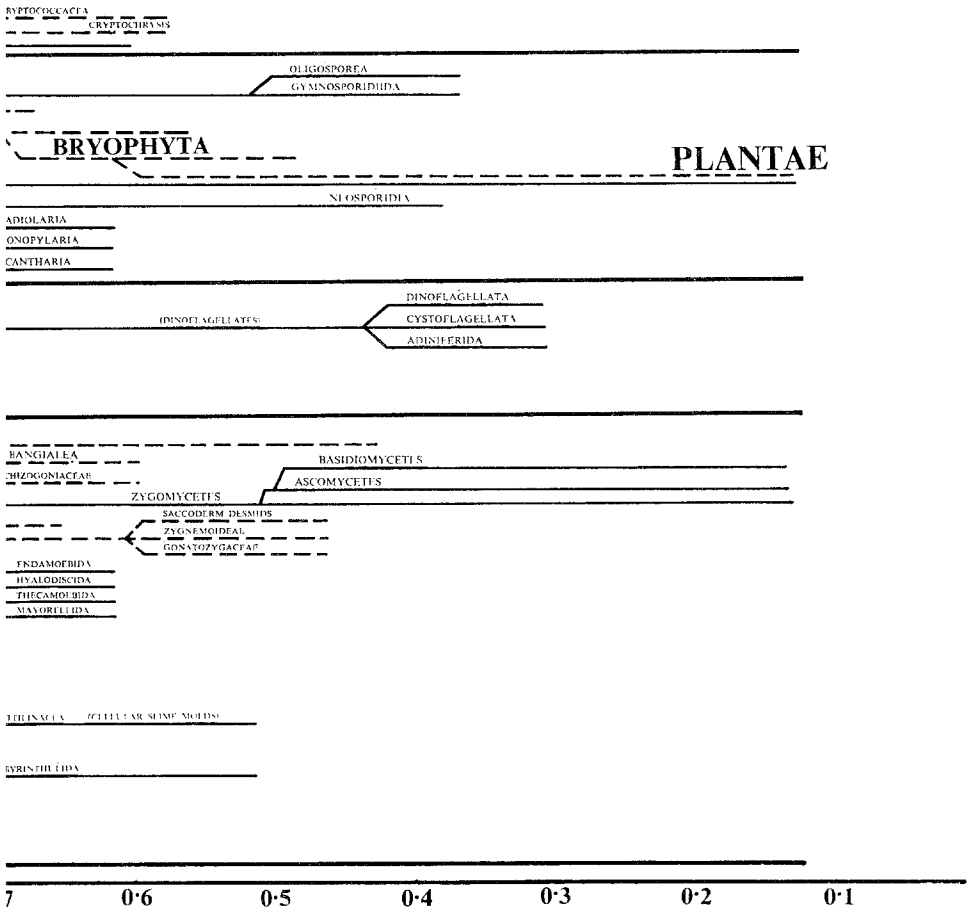


FIG. 1. The evolution of the eukaryotic cell.



Phylogenetic tree of the Protoctista Kingdom.

[*facing p. 228*

contain mitochondria and are fundamentally aerobic. Based on the abundance of fossil eukaryotes, it must have occurred before the dawn of the Cambrian. Hence, the most likely period for the evolution of mitosis is between $1{\cdot}2$–$0{\cdot}6 \times 10^9$ years ago—a period of about 600 million years.

The first symbiotic acquisition, that of the protomitochondrion, produced cells with the typical eukaryotic carbohydrate oxidative pathways. The anaerobic breakdown of glucose to pyruvate along the Embden-Myerhof pathway occurred in the soluble cytoplasm under the direction of the host genome. Further oxidation of glucose using molecular oxygen *via* the Krebs cycle (H atoms from organic acids combine with DPN, FAD, and cytochromes; ATP is generated; and water is eliminated) occurred only in the symbiotic mitochondrion under the direction of its own genes.

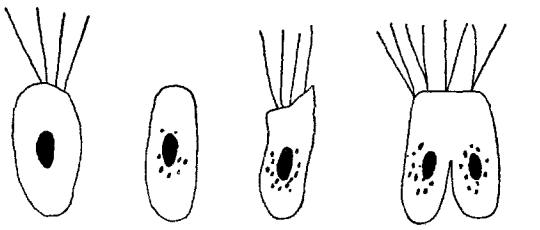
This mitochondrial-host symbiosis may have resulted in the typical eukaryotic phospholipid membrane and steroid synthesis and, in particular, the formation of a nuclear membrane and endoplasmic reticulum. The greater amounts of energy available after the incorporation of the mitochondrion resulted in large cells with amoeboid and cyclotic movement. However, the diversity in types and amounts of proteins such cells could make would have been limited by the amount of DNA available to administer protein synthesis. Hence, in the absence of any efficient mechanism to insure the equal distribution of newly synthesized DNA, the size of the amoeboids host cell must have been limited. Multinuclearity and duplicated cistrons or entire genomes (polyploidy) may have been early mechanisms to distribute greater amounts of DNA evenly to daughter cells, since multiple copies greatly increase the probability that each daughter will get at least one copy of the genome. However, the inefficiency of polyploidy and the selective advantage of linkage groups over polyploidy have been demonstrated (Gabriel, 1960). The problem of equal segregation of the daughter linkage groups remained.

2.4. THE EVOLUTION OF MITOSIS IN AMOEBOFLAGELLATES

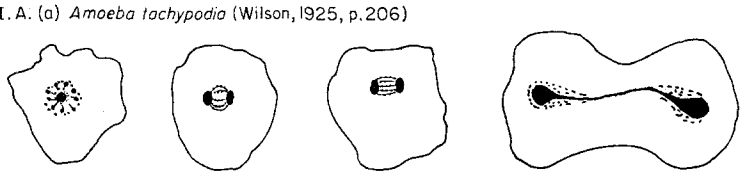
How did the efficient mitotic mechanism which equally segregates daughter linkage groups evolve? The dividing nuclei of the lower eukaryotes themselves suggest a plausible evolutionary sequence for mitosis.

It is likely that the large size and heterotrophic habits of the amoeboids led to the ingestion of all kinds of small prokaryotes (Trager, 1964). Among these, it is hypothesized that some ancestral amoeboid acquired a motile parasite, perhaps spirochaete-like, by ingestion. The genes of the parasite, of course, coded for its characteristic morphology, $(9+2)$ fibrils in cross section. Amoeboid host cells which retained their endosymbionts had the

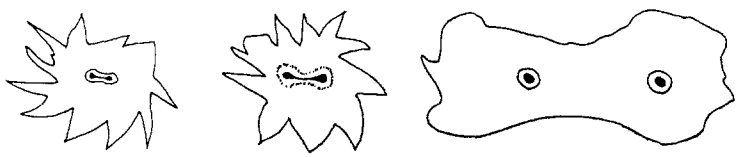
I. *Tetramitus* (Wilson, 1925, p. 82)



II. A. (a) *Amoeba tachypodia* (Wilson, 1925, p. 206)



II. A. (b) *Amoeba polypodia* (Wenyon, 1926, p. 63)



II. A. (c) *Hartmanella hyalina* (Wenyon, 1926, p. 63)

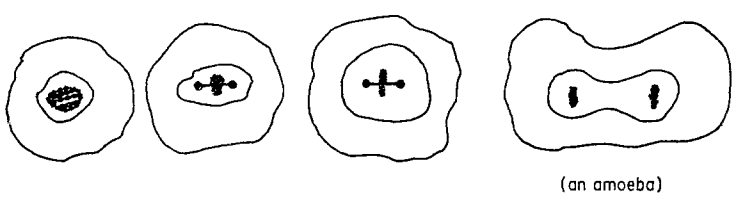


FIG. 2. Mitotic figures of some representative eukaryotes. These figures were adapted from the illustrations which may be found in the references cited. The names of the organisms given are generic. The name in parenthesis indicates the larger group in which the organism can be found in Fig. 1, the phylogenetic tree. A common description is given for some of the more obscure genera. The Roman numeral groups correspond to branches on the phylogenetic tree as discussed in sections 2.5.1 to 2.5.6 of the text. The organisms in groups I, III and V are thought to be premitotic. In groups II, IV and VI the subgroupings A and B refer to "premitotic" and "eumitotic", respectively. [The lower case letters (a), (b) . . . are simply included for reference.]

immense selective advantage of motility long before mitosis evolved; they could actively pursue their food.

By hypothesis, the motile prokaryotic endosymbiont itself is the ancestor of the complex flagellum of eukaryotic cells. The replicating nucleic acid of the endosymbiont genes (which determines its characteristic (9+2) substructure) was eventually utilized to form the chromosomal centromeres and centrioles of eukaryotic eumitosis and to distribute newly synthesized host nuclear chromatin to host daughters.

How did endosymbiont nucleic acid differentiate into chromosomal centromeres and centrioles? At least two series of mutational steps were required: one leading to the development of some attraction between nucleic acid of the host and that of the symbiont, and eventually, to permanent connections of daughter endosymbionts precisely to daughter chromosomes of the hosts in the formation of chromosomal centromeres; a second leading to the segregation of the replicated daughter endosymbionts to opposite poles of the host cell. In any case, in each generation only those amoeboid daughter cells containing a full euploid genome—that is, at least one copy of each gene—were selected. This insured continuing selection pressure for improved mechanisms of host nucleic acid segregation. Judging from present-day mitotic figures among lower eukaryotes, it probably led to the development of eumitosis at various times in various primitive amoeboflagellates. This might be considered analogous to the development of different mechanisms determining sexual polymorphism in higher organisms. The biological goal of all of these is the same: insurance that genetic recombination systematically accompanies reproduction. Just as the various sex determining mechanisms are not directly homologous (e.g. ovaries in insects, mammals, and flowering plants), it is likely, too, that some eumitoses in lower eukaryotes are analogous rather than directly homologous.

A corollary of this is the assertion that primitive eukaryotes can be classified according to the various lines they represent in the evolution of eumitosis. An attempt to reconstruct these lines on the basis of cytological and genetic information accumulated from the literature is presented in sections 2.5.1 to 2.5.6 below. The plausible steps in the differentiation of the chromosomal centromeres and centrioles from "(9+2) homologue" flagellar basal bodies have been reconstructed from the available data and the immediate selective advantages of each step is discussed in sections 2.5.1 to 2.5.6. The large groups of organisms which presumably derived from these steps, are presented on branches I to VI of the phylogenetic tree (Fig. 1). The groups were formed to include as much data as possible on the eumitosis of lower eukaryotes in a way which is considered to be consistent with the general classification of these organisms, *exclusive of their photosynthetic plastids.*

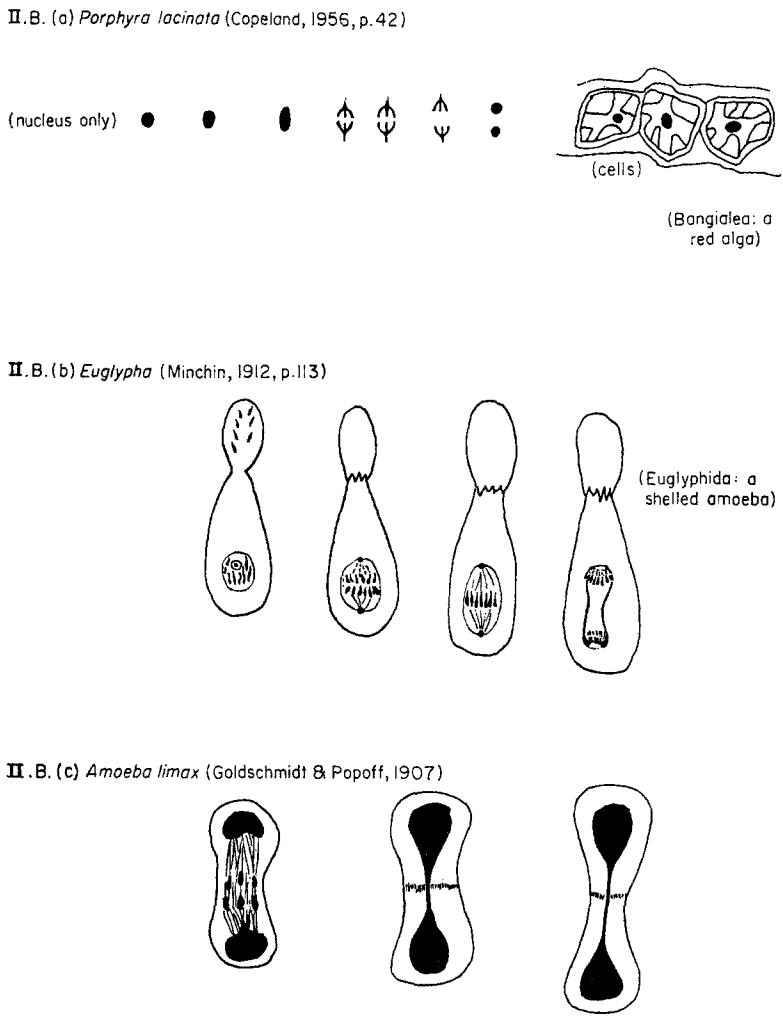


FIG. 2. Mitotic figures of some representative eukaryotes (*cont.*).

2.5 STEPS IN THE EVOLUTION OF EUMITOSIS

The corresponding figures, together comprising "Fig. 2", were adapted from the literature as examples of actual mitotic figures. The term "(9+2) homologue" refers to the flagella and cilia, as well as to the more specialized cirri (fused cilia), axopodia, and other cellular organelles with the characteristic electron microscope cross section. "Basal bodies" (found at the base of these motile organelles, maybe self-reproducing), centrioles, or some other submicroscopic homologues are considered to be the repository of the nucleic acid of the replicating system and, thus, the descendants of the genes of the original symbiont. The "division center" is considered to be homologous also (Wilson, 1925, see p. 204). Therefore, the term "(9+2) homologue" is used here in its most general sense; it implies the genetic system that codes for the development of flagella, cilia, "division centers", centrioles, or any other of these homologous organelles. Diagrams I to VI, which illustrate the hypothesized relationships between host genome and (9+2) homologue genome, include in each generation the ratios of symbiont to host genomes suggested by the theory. Although at least one dividing (9+2) homologue on each host linkage group (i.e. a chromosomal centromere) is required to insure euploidy, multiple numbers of chromosomes in the schematic diagrams have been omitted for clarity; their omission changes in no way the general argument. We know that very closely related species can differ in their total chromosome number and, in general, the number *per se* of any fundamentally replicative structure itself is a very poor criterion in taxonomy (Dillon, 1962). In the diagrams, the numerator in the ratio "symbiont (9+2) genomes per host nuclear genome" refers only to *functionally* distinct (9+2) homologues.

"Premitosis", in Diagrams I to VI, explicitly illustrates the phenomena presumably at the basis of the lack of "sex" (meiosis and fertilization) in many lower eukaryotes. These organisms have the general features of eukaryotic cells (mitochondria, nuclear membranes, etc.), but their "aberrant" division figures often lack the spindles and centrioles of typical eumitosis. (*Amoeba*, *Euglena*, *Tetramitus*, etc.: see Fig. 2). They are probably not degenerate phytoflagellates, but eukaryotic organisms which are premitotic in the sense that they branched off the main lines of higher cell evolution before eumitosis evolved. The diploid-haploid cycle (i.e. "sex") is the surest indication of the evolution of eumitosis in any group of organisms. Eumitotic organisms, therefore, are those which demonstrate the typical genetic patterns described as Mendelian.

2.5.1. (9+2) *Homologue as basal body for flagella only*

In the first group of primitive eukaryotic flagellates, the nucleic acid of the motile symbiont is used only for its own replication and synthesis of

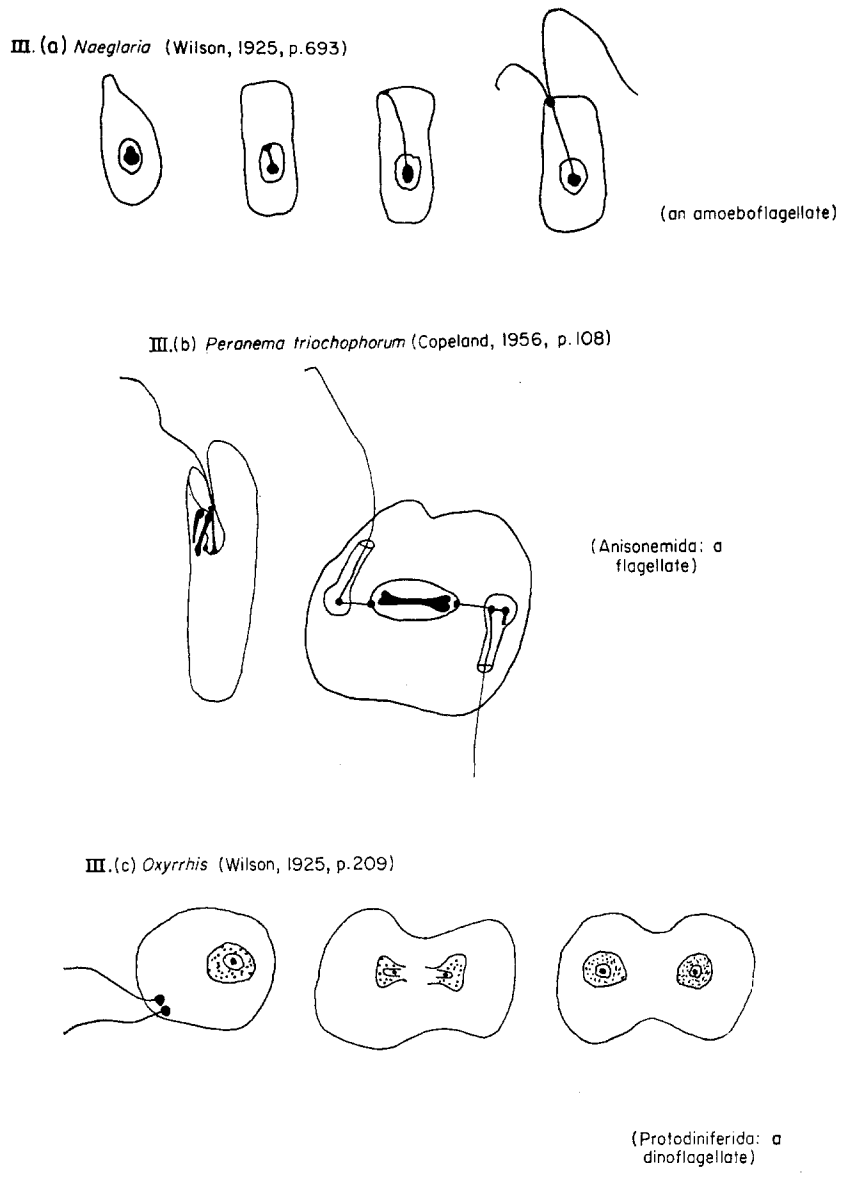


FIG. 2. Mitotic figures of some representative eukaryotes (*cont.*).

its own protein. The immediate selective advantage of the acquisition of the (9+2) endosymbiont was motility. Relic of this event may persist in some isolated groups of small flagellates, e.g. *Tetramitus* (Wilson, 1925, see p. 82.) "Mitosis" in these organisms is by no means standard and, of course, sexuality in the meiotic sense is unknown and, by hypothesis, will never be found (Fig. 2. I, Diagram I).

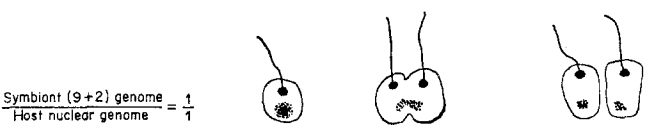


DIAGRAM I

2.5.2. (9+2) *Homologue incorporated into the nucleus for division center, no flagella*

Mutations occurring in populations of organisms described in section 2.5.1, led to attractions between host and (9+2) endosymbiont nucleic acid, which resulted in the incorporation of the replicating genome of the (9+2) symbiont into the nucleus of the host. When the (9+2) symbiont divided, it was utilized by the host as an intranuclear "division center". (The term "division center" is taken from the classical literature, see Wilson, 1925, p. 204.) The body stains deeply with nuclear dyes. Its homologies to basal bodies of flagella, etc., were recognized (and disputed) in the elegant light-microscopic studies on protozoan mitosis early in this century (Wilson, 1925, p. 206; Wenyon, 1926, see pp. 62 and 102).

These events first produced premitotic amoebae, which never have flagella at any stage of their life cycle but contain intranuclear division centers. Possibly multinuclear and other asexual amoebae are relics of this premitotic stage. For example, Dobell says of division in *Amoeba lacertae*: "No equatorial plate is formed and the 'chromosomes', or chromatic granules wander irregularly toward the poles, the whole karyosome meanwhile drawing out into a spindle shape and finally dividing. It is doubtful whether we can here speak of chromosomes or even mitosis, but such a type of division might well form the point of departure for the evolution of a true mitotic process" (Wilson, 1925, p. 213; Wenyon, 1926, p. 101) [Fig. 2. II A. (a) to (c)].

Eventually different groups of eumitotic organisms evolved from these various amoebae, namely, some eumitotic amoebae; the zygomycetes, ascomycetes, and basidiomycetes; the cellular slime molds; the conjugating green algae; the two great classes of red algae and their green counterparts

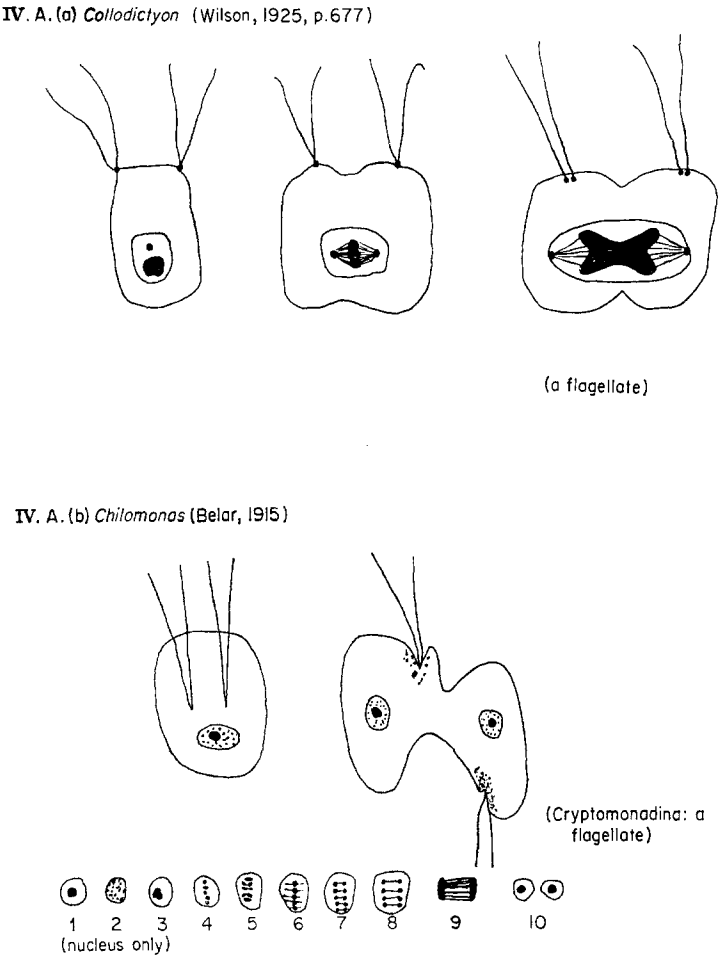


FIG. 2. Mitotic figures of some representative eukaryotes (*cont.*).

—the *Schizogoniacea.* All of these organisms lack flagella at all stages in their life cycles, even though many of them are aquatic [Fig. 2. IIB. (a) to (c)] (Copeland, 1956, p. 42; Minchin, 1912, p. 113; Goldschmidt & Popoff, 1907).

How did eumitosis begin in this line which permanently lacked flagella? The first step may have involved replications of the (9+2) homologue (after it had become the intranuclear division center) in the absence of nuclear division. This made available *pairs* of intranuclear (9+2) homologues. Some mutation then occurred causing one member of the pair to be attracted to host chromatin and eventually to function as chromosomal centromere. The other, not attracted to host chromatin, functioned as an intranuclear centriole. The members of these pairs attracted one another. The attraction of the (9+2) homologue (chromosomal centromere) dragging with it host chromatin as it moved toward its sister (9+2) homologue (intranuclear centriole) may be analogous to whatever mechanisms are generally involved in attractions of prokaryotic cells to each other prior to mating.

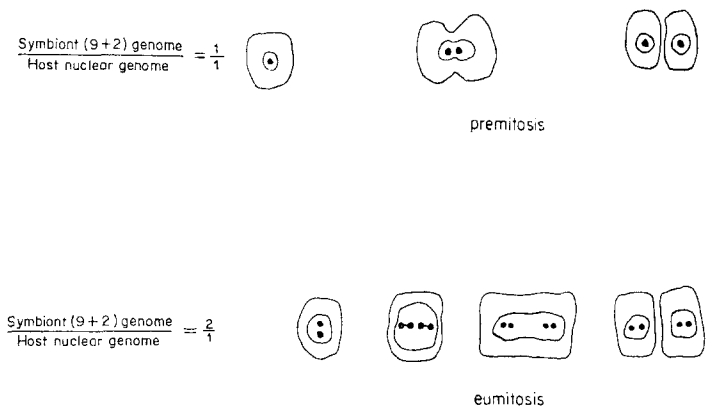
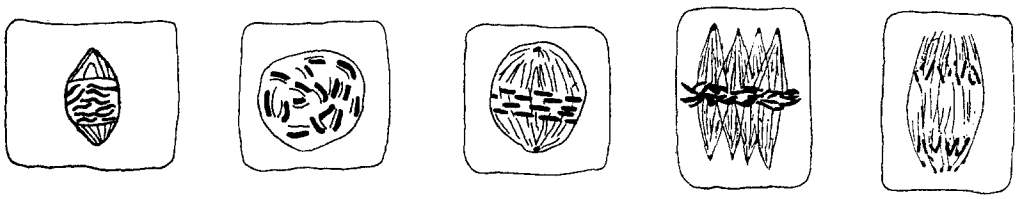


DIAGRAM II

2.5.3. (9+2) *Homologue used both as intranuclear division center and as flagellar basal body*

Off of this ancestral animal flagellate line, cells evolved which were motile at certain stages in the life cycle. At other stages the (9+2) flagellar basal body entered the nucleus and divided. While the (9+2) homologue divided, it was "borrowed" as an intranuclear division center for segregation of host chromatin. After division, the (9+2) homologue resumed its function at the base of the flagellum. This line may have led to a number of amoebo-flagellates which do not divide during their motile stages; in some a morphological relationship between flagella and nucleus may be seen [Fig. 2. III (a) to (c)].

IV. B. (a) *Allium* (Wilson, 1925, p. 697)



(Plantae: onion)

IV. B. (b) *Paramecium caudatum* (Wilson, 1925, p. 610)

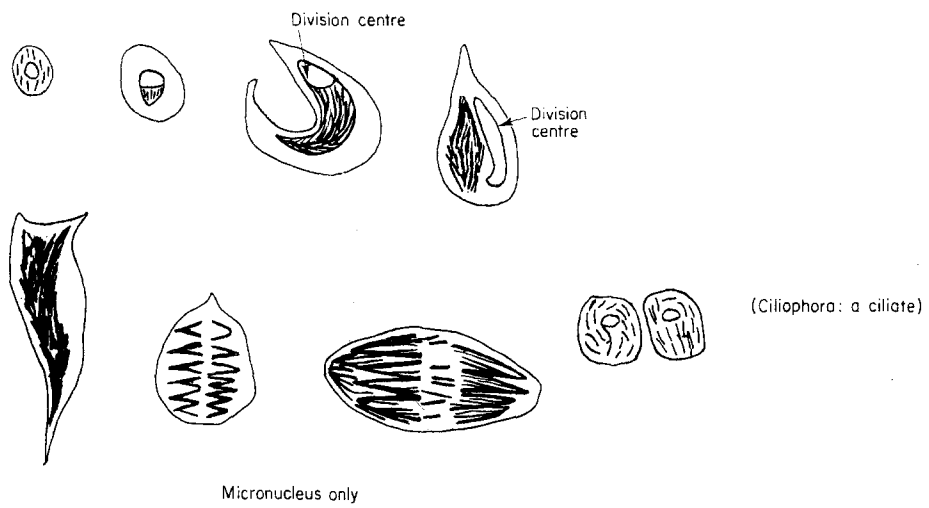


FIG. 2. Mitotic figures of some representative eukaryotes (*cont.*).

Relics of this line are, for example: *Naeglaria* (Wilson, 1925, p. 693), *Anisonemids* (peranemids) (Copeland, 1956, p. 108), and dinoflagellates (Wilson, 1925, p. 209); *Dimastigamoeba*, *Mastigella* and *Mastigina* (Goldschmidt, 1907). The first three groups are considered to be premitotic in that sexual phenomena are not known, or its occurrence seriously disputed (Diagram III).

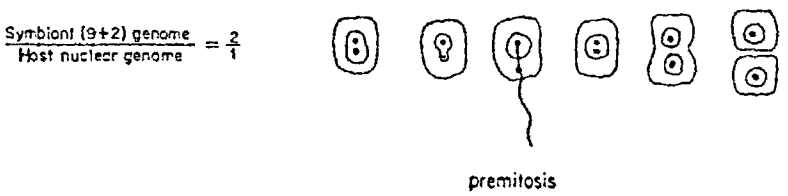


DIAGRAM III

2.5.4. (9+2) *Homologues used as basal bodies to flagella and other* (9+2) *homologues permanently differentiated as intranuclear division centers*

Mutations occurring off the general line of amoeboflagellates essentially produced two separate (9+2) homologue "clones". One mutant (9+2) homologue produced "offspring" which served permanently as intranuclear division centers. The other (9+2) homologue produced offspring which remained as basal bodies to the flagella only. An intranuclear division center and eventually eumitosis evolved in a line in which (9+2) homologue-basal bodies to independent flagella were retained. This series of mutations (which may have occurred more than once) presumably led to groups of protozoans, green algae, golden algae, etc. Some of these groups are clearly premitotic (e.g. *Euglenids*) [Fig. 2. IV (a), (b)], (Wilson, 1925, p. 697; Belar, 1915).

One, or perhaps several, of these lines led to the eumitotic green plants with anteriorly flagellated motile stages and (by hypothesis) intranuclear division centers (Wilson, 1925, p. 151); another may have evolved some sporozoans (Copeland, 1956, p. 17).

The selective advantage of these mutations is clear: they insure the organism both equal distribution of its chromatin and retention of its flagellar motility.

The relatively homogenous groups of protozoans, the ciliates with their dimorphic nuclei, presumably evolved from an analogous series of mutations off the primitive amoeboflagellate line. They have a eumitotic micronucleus (with an intranuclear spindle) reserved for genetic continuity (Weinrich, 1954). They also maintain replicating basal bodies to the (9+2) cilia of the cortex independent of the mitotic apparatus. However, the mitotic "germ line" and premitotic "soma" are strangely differentiated in the two types of nuclei in these organisms. The endopolyploid macro-

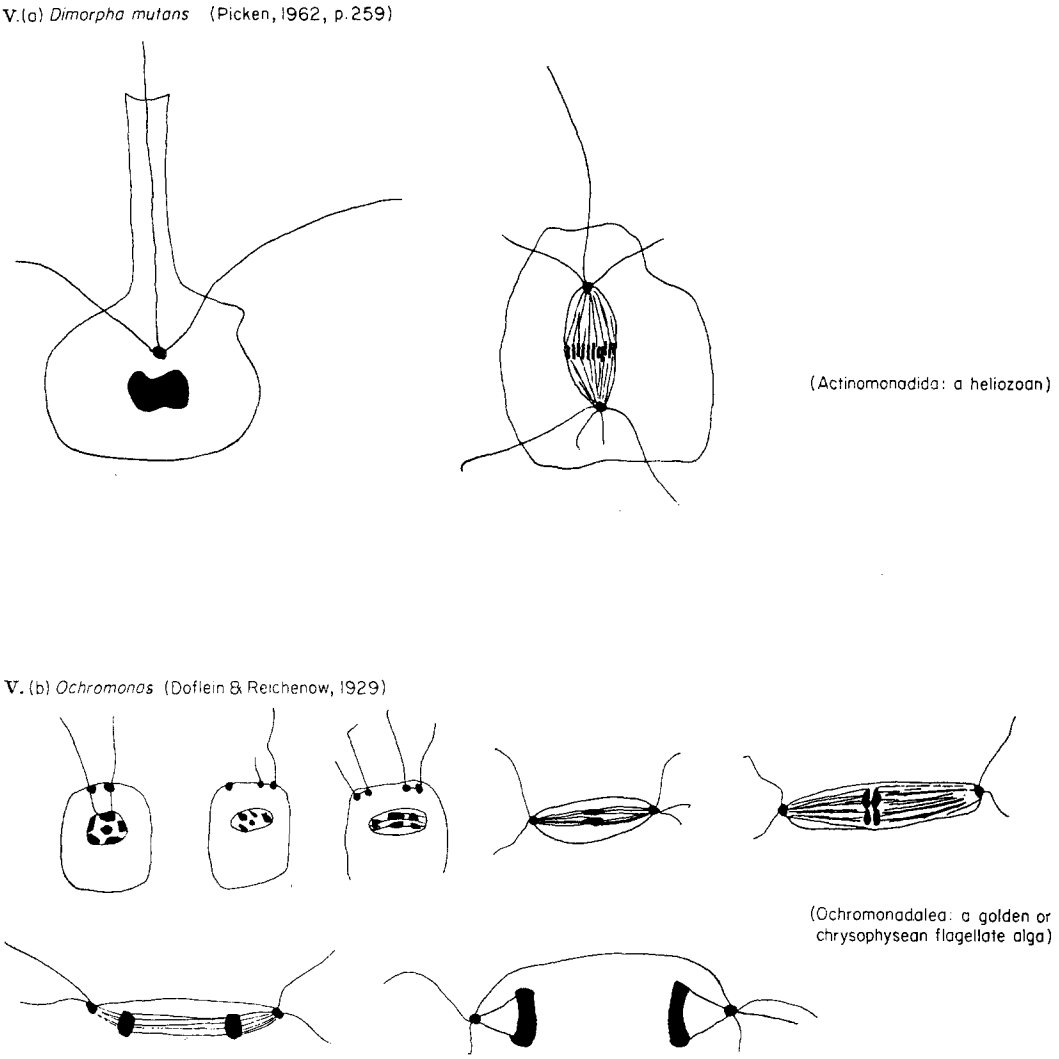


FIG. 2. Mitotic figures of some representative eukaryotes (*cont.*).

nucleus contains no chromosomal centromeres or individual chromosomes and divides by amitosis. Presumably, the series of mutations which were selected for in the evolution of eumitosis in the micronucleus began in a binucleate cell, one in which many copies of the genome were regularly produced and reserved for the active administration of protein synthesis. The evolution of eumitosis never occurred in the macronucleus [Fig. 2. IV B. (b)] (Wilson, 1925, p. 610).

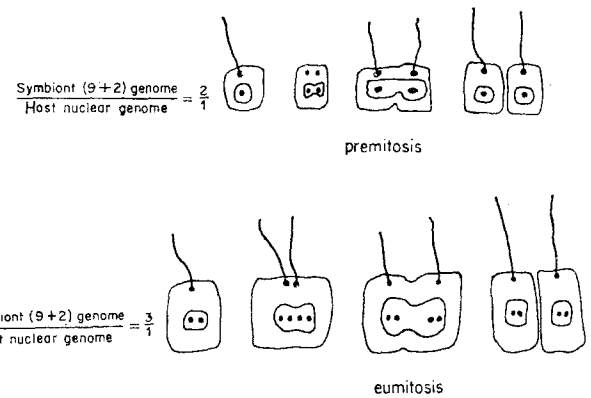


DIAGRAM IV

2.5.5. (9+2) *Homologue used both as basal body to flagella and as extranuclear "division center"*

In members of the early amoeboflagellate populations already containing intranuclear (9+2) homologues mutations must have occurred leading to cells in which the basal bodies of the flagella, when they divided, functioned as extranuclear division centers. Such striking examples of the basal body functioning as an extranuclear division center during mitotic stages exist in quite different forms among many extant organisms [*Dimorpha mutans* (Picken, 1962), *Clathrina* (Wilson, 1925, p. 42), *Ochromonas* and *Centropyxis* (Doflein & Reichenow, 1929)]. Some of these organisms [e.g. *Acanthocystis* (Calkins, 1909), *Wagnerella* (Wilson, 1925, p. 677)] have animal-type division figures, but ones in which centrioles are indistinguishable from the flagellar basal bodies. During division the flagella of many of these organisms are still attached to the centrioles at the poles of the mitotic figure. In some cases when the (9+2) homologue divides, one product of the division functions as centriole, and the other gives rise to the flagellar basal body which differentiates and is no longer capable of continued replication (Renaud & Swift, 1964).

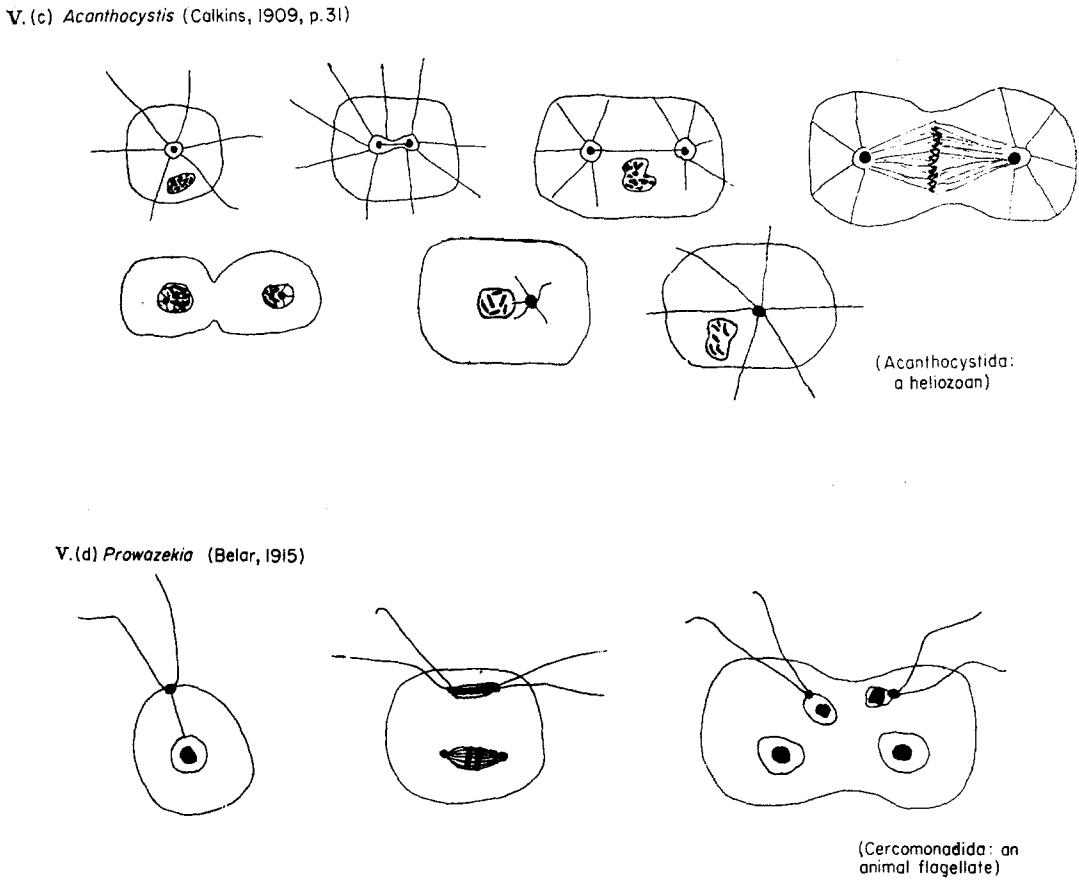


FIG. 2. Mitotic figures of some representative eukaryotes (*cont.*).

On this evolutionary line, in which dividing extranuclear basal bodies functioned as centrioles attracted to intranuclear chromosomal centromeres, it is likely that a series of specialized flagellates arose, which maintain throughout the life cycle some morphological connection between the flagellar apparatus and the mitotic figure, e.g. *Trichomonas* (Wilson, 1925, p. 205); *Polymastix* (Wilson, 1925, p. 694); *Heteromita* (Wenyon, 1926, p. 118); *Prowazekia* (Belar, 1915) and *Eudorina* (Hartmann, 1921). In some organisms this connection was probably secondarily lost, e.g. *Cryptobia* (Copeland, 1956, p. 160); *Herpetomonas* (Wenyon, 1926, p. 118); *Vaucheria* (Fritsch, 1935, p. 70); *Dictyota* (Wilson, 1925, p. 200); *Paramoeba* (Goldschmidt & Popoff, 1907); *Gurleya* (Wenyon, 1926, p. 742). Hypermastigote protozoans, whose mitotic centrioles form from the flagellar band in the living cell, are especially illustrative of this group (Copeland, 1956, p. 171; Cleveland, 1956, 1963). For example, after much study of the life cycle of the centrioles in the hypermastigote, *Barbulanympha*, Cleveland concludes:

". . . the reorganization process (of the centrioles) just described shows several things clearly; a definite relationship between the hypermastigote centrioles and those of higher forms of life; the ability of the centriole at certain times to function more than once in the formation of flagella axostyles, and parabasals, just as it is able to do at all times in the formation of the achromatic figure (i.e. mitotic apparatus); the ability of the centrioles to function in the production of extranuclear organelles without reproducing themselves, and also without accompanying nuclear or cytoplasmic reproduction; the inability of flagella, parabasals and axostyles to reproduce themselves; and most important of all, the fact that the anterior tip of these unusually large centrioles of flagellates is their reproducing portion" (Cleveland, 1956).

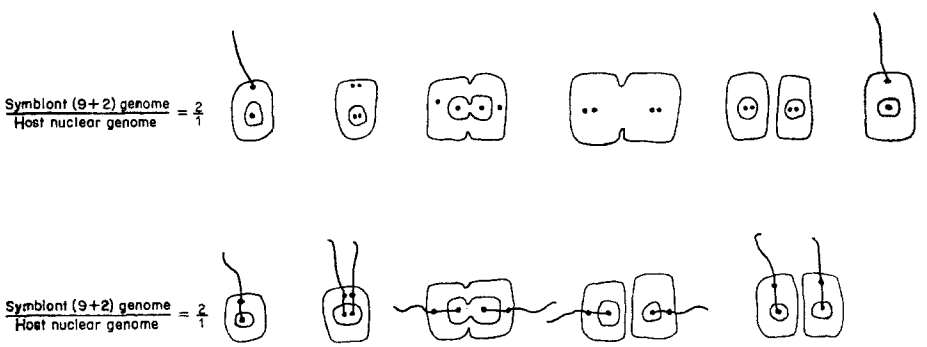


DIAGRAM V

2.5.6. (9+2) *Homologues used as basal bodies to flagella and other* (9+2) *homologues permanently differentiated as extranuclear division centers (centrioles)*

This series of mutations leading to the typical centrioles and amphiasters of animal-type mitosis involve steps analogous to those in section 2.5.4. Two "clones" of (9+2) homologues were produced: in one, the (9+2) homologue permanently gives rise to basal bodies which only gives rise to other basal bodies which produce flagella. In the other, the (9+2) homologue gives rise to extranuclear centrioles. This may have involved mutations leading to the loss of the two central fibers of the (9+2) homologue leading to the non-motile (9+0) centriole structure. [It may be the central fibers confer motility on the flagella and are somewhat analogous to the axial fiber of spirochaetes (Stanier *et al.*, 1963, p. 158).] The selective advantage of a permanent functional differentiation of the two organelles is clear, as in section 2.5.4, flagellar motility is completely independent of the mitotic apparatus: flagellated cells, in eumitotic organisms, too, can divide. Examples of this type of mitosis with amphiasters surrounding the extranuclear centrioles are found in some protozoans, and most eumetazoan groups (Wilson, 1925, p. 124). The conspicuous extranuclear centriole of the diatoms may be functionally homologous, too. This is consistent with the interesting late arrival of this relatively homogeneous group of advanced algae on the fossil scene, during the Cretaceous [Fig. 2. VI (b)] (Fritsch, 1935, p. 660).

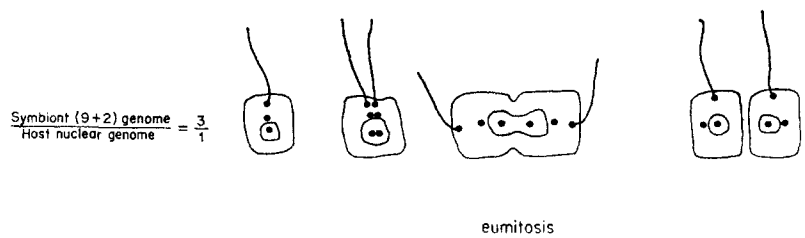
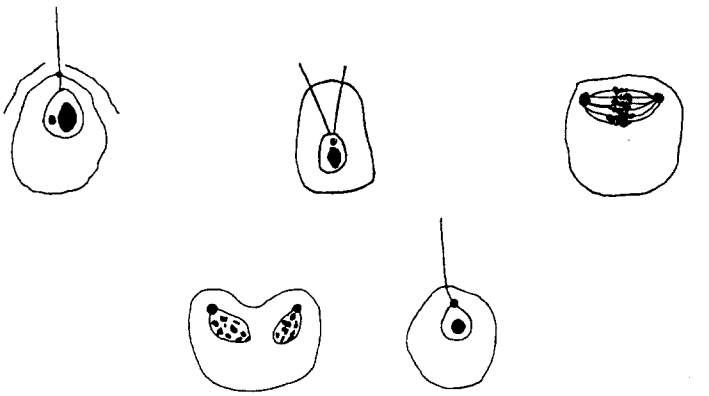


DIAGRAM VI

2.6. THE EVOLUTION OF EUKARYOTIC PLANTS FROM VARIOUS LINES OF PROTOZOANS WHICH ACQUIRED SYMBIOTIC PROKARYOTIC ALGAE

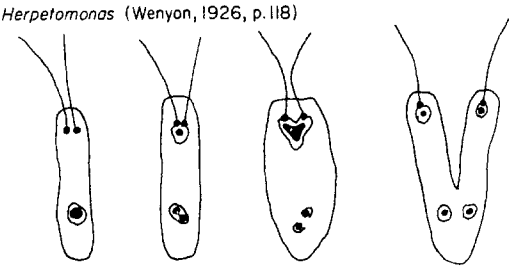
As outlined above, the evolution of mitosis is assumed to have occurred millions of years after the evolution of photosynthesis. This hypothetical origin of eukaryotes is completely incompatible with the notion that a simple phytoflagellate is the ancestor to extant higher cells—eukaryotic plant cells did not evolve oxygen-eliminating photosynthesis which later "packaged" into membrane-bounded plastids; they acquired it by sym-

V.(e) *Eudorina* (Hartmann, 1921)



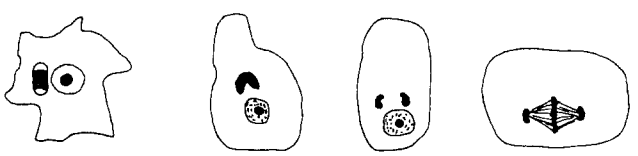
(Volvocales: a green alga)

V.(f) *Herpetomonas* (Wenyon, 1926, p. 118)



(Oikomonadacea: an
an animal flagellate)

V.(g) *Paramoeba eilhardi* (Goldschmidt & Popoff, 1907)



(Paramoebida: an
animal flagellate)

FIG. 2. Mitotic figures of some representative eukaryotes (*cont.*).

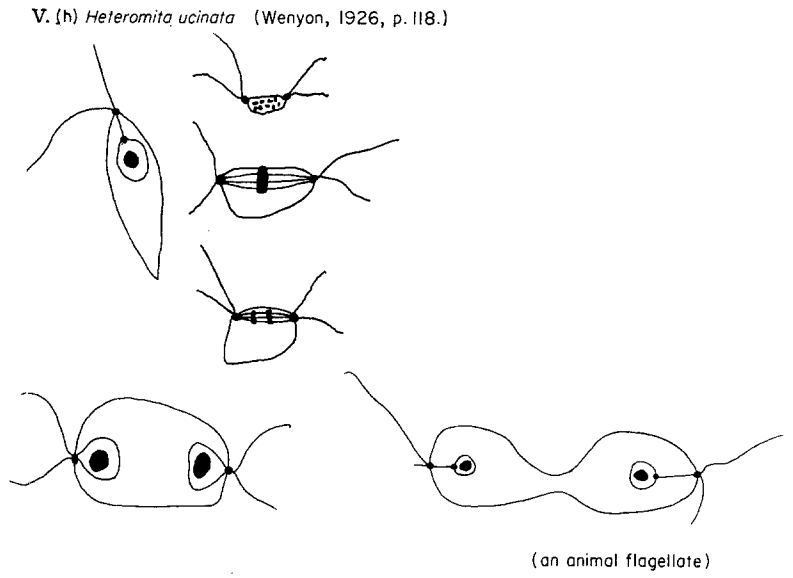


FIG. 2. Mitotic figures of some representative eukaryotes (*cont.*).

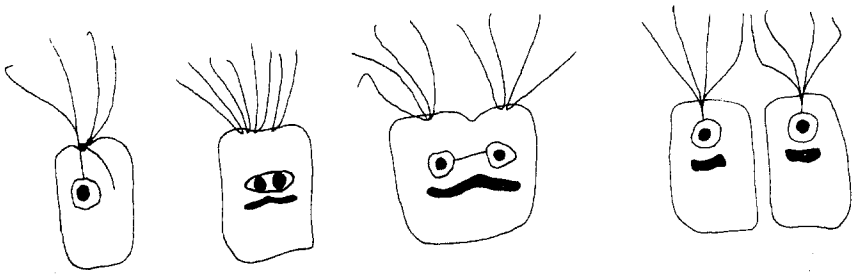
biosis. The diversity of cell structure and the life cycle in lower eukaryotic algae imply that different photosynthetic prokaryotes (protoplastids) were ingested by heterotrophic protozoans at various times during the evolution of eumitosis. The protoplastids themselves evolved from oxygen-eliminating prokaryotes, homologous to blue-green algae. To their own selective advantage they remained in the protozoan hosts which had ingested them and eventually became the obligately symbiotic plastids, retaining their characteristic photosynthetic pigments and pathways. In many eukaryotic algae and all higher plants, of course, the symbiosis continued to evolve a great deal, and vestiges of heterotrophy were eventually lost. This implies that in eukaryotic plants the plastids are homologous to blue-green algae; the non-chloroplast part of plants is directly homologous to eukaryotic heterotrophs.

The Phylogenetic Tree—Fig. 1. Based on the above hypothesis, Fig. 1 presents the major groups of eukaryotic lower organisms on a phylogenetic tree. In an extremely original taxonomic treatise, H. F. Copeland classified many of these lower eukaryotic organisms into relatively isolated natural groups. Except for accepting genetic autonomy of photosynthetic plastids, Copeland's independent work is remarkably consistent with the evolutionary theory presented here. His book (Copeland, 1956) was invaluable to the development of this phylogeny.

Thus, there are two major innovations presented in the phylogenetic tree. One involves the separation of lower eukaryotic algae into groups based on the supposed homologies of the "host" or protozoan part of the organism. The lettered circles representing plastids on the lines indicate the homologies of the prokaryotic photosynthetic endosymbiont; with less accuracy, the positions of these circles represent the stage in evolution at which the symbiotic acquisition occurred. The second is an attempt to reclassify admittedly heterogeneous groups of protozoans (e.g. sporozoans, sarkodina, etc.) according to their mitotic cytologies and, hence, the stages they presumably represent in the evolution of eumitosis. Aside from these major modifications, standard taxonomy has been followed. Copeland's terminology is adapted on the phylogenetic tree for all organisms except the green algae. The terminology of Fritsch is used for these. Except for organisms explicitly mentioned elsewhere on the tree, the groups are considered to be equivalent to those of Copeland.

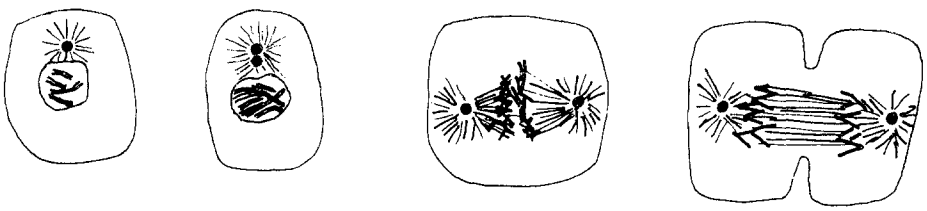
The acquisition of photosynthesis by eukaryotic heterotrophs then, may be thought of as quite analogous to some recognized symbioses, which on the basis of the identification of the photosynthetic symbiont, must have originated much more recently, e.g. *Paramecium bursaria*, chrysophytes in Radiolarians, *Hydra viridis*, the algal-fungal associations in lichens. Occur-

V. (j) *Polymastix* (Wilson, 1925, p. 205)



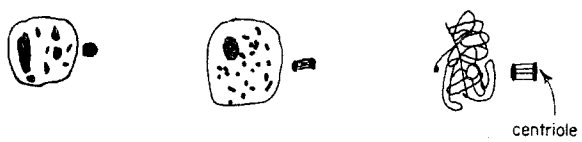
(Polymastigida: an animal flagellate)

VI. (a) *Ascaris* (Wilson, 1925, p. 124)



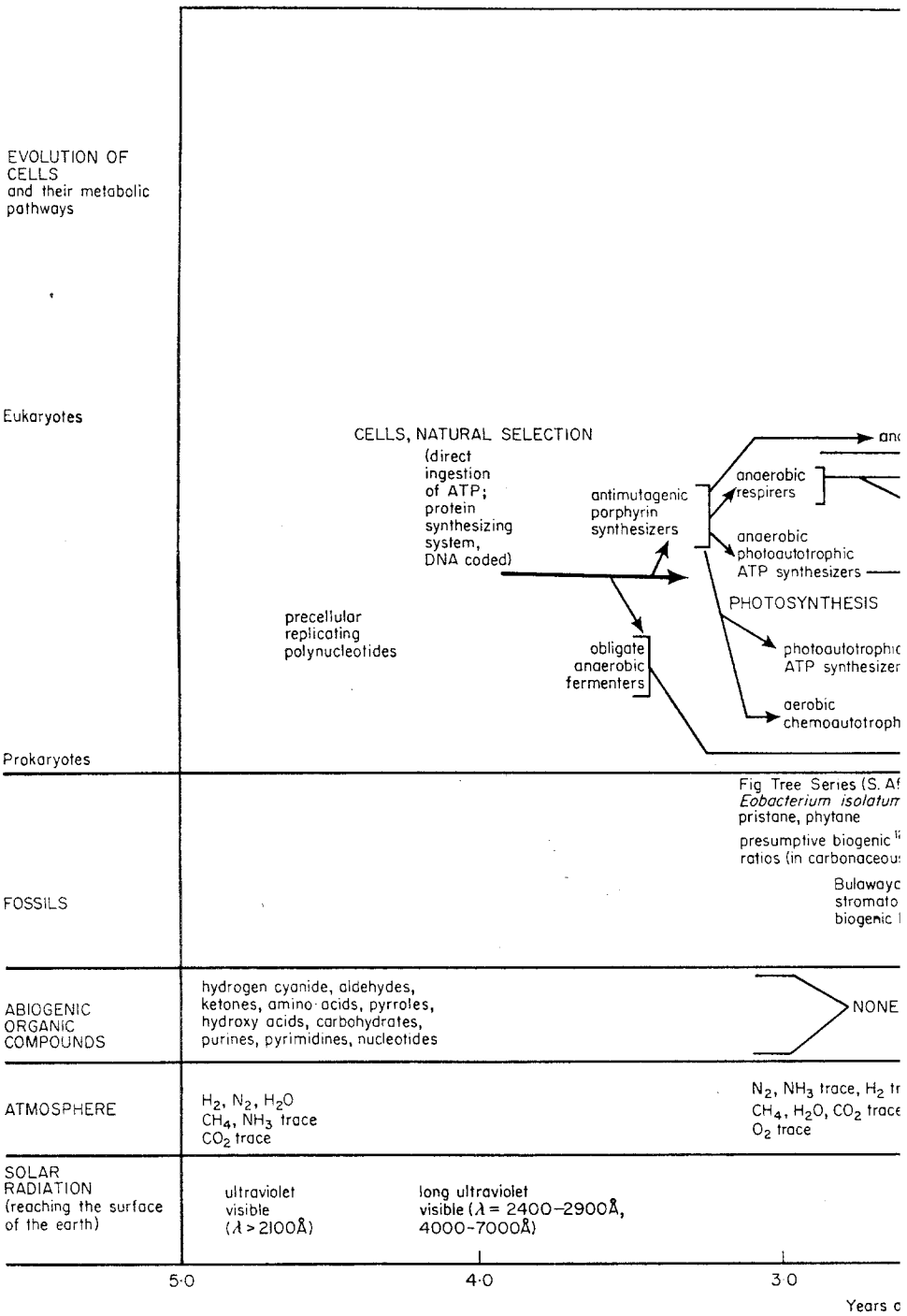
(Animalia: a roundworm)

VI. (b) *Surirella* (Fritsch, 1935, p. 660)

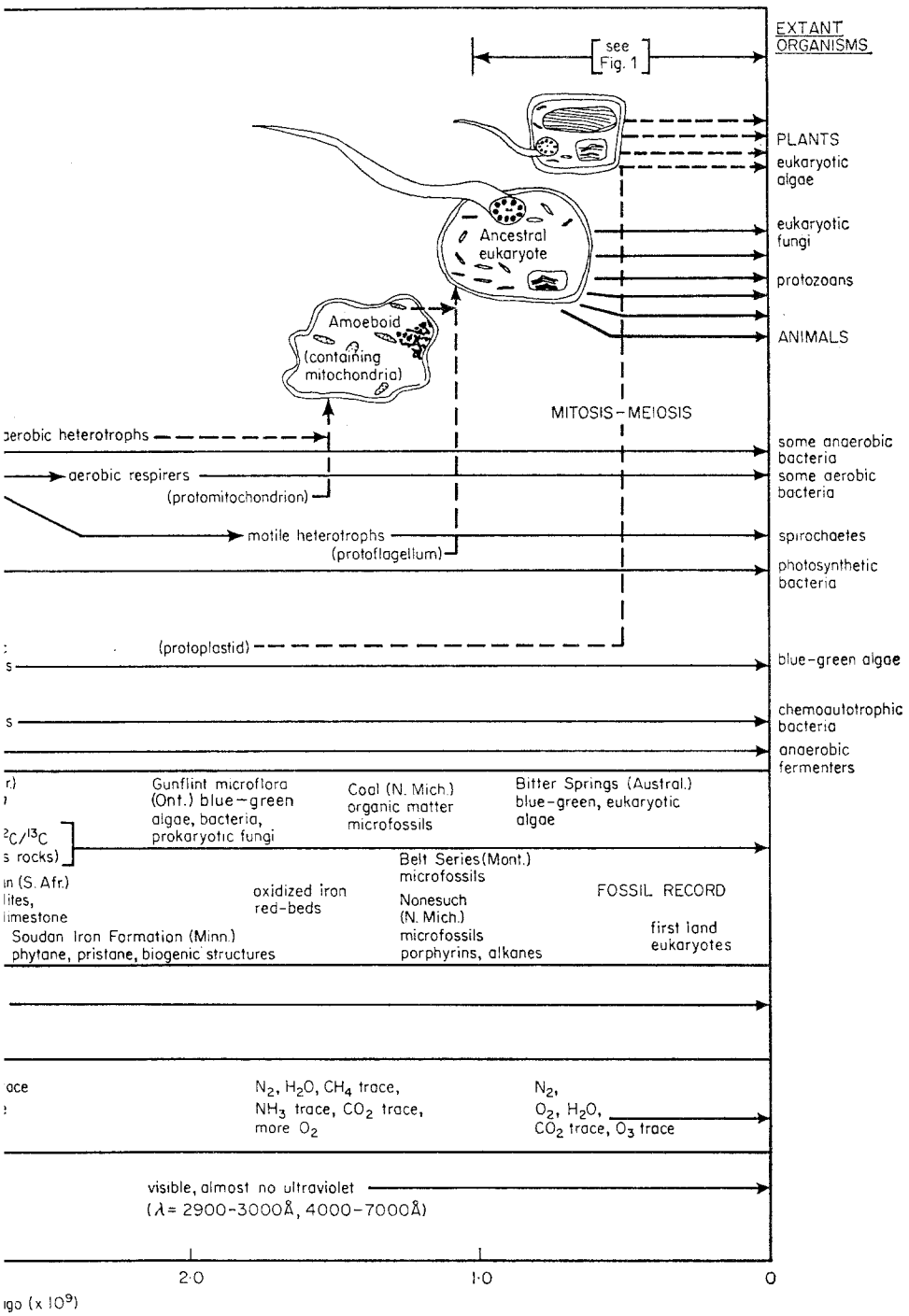


(Bacillaracea: a diatomaceous alga)

FIG. 2. Mitotic figures of some representative eukaryotes (*cont.*).


EVOLUTION OF CELLS and their metabolic pathways
Eukaryotes
CELLS, NATURAL SELECTION
(direct ingestion of ATP; protein synthesizing system, DNA coded)
antimutagenic porphyrin synthesizers
anaerobic respirers
anaerobic photoautotrophic ATP synthesizers
PHOTOSYNTHESIS
precellular replicating polynucleotides
obligate anaerobic fermenters
photoautotrophic ATP synthesizer
aerobic chemoautotroph
Prokaryotes
Fig Tree Series (S. Af
Eobacterium isolatum
pristane, phytane
presumptive biogenic
ratios (in carbonaceou
Bulawayc
stromato
biogenic
FOSSILS
ABIOGENIC ORGANIC COMPOUNDS
hydrogen cyanide, aldehydes, ketones, amino-acids, pyrroles, hydroxy acids, carbohydrates, purines, pyrimidines, nucleotides
NONE
ATMOSPHERE
H2, N2, H2O
CH4, NH3 trace
CO2 trace
N2, NH3 trace, H2 tr
CH4, H2O, CO2 trace
O2 trace
SOLAR RADIATION (reaching the surface of the earth)
ultraviolet
visible
(λ > 2100Å)
long ultraviolet
visible (λ = 2400–2900Å, 4000–7000Å)
5·0
4·0
3·0
Years c

ɜLE 1



[facing p. 248

rences of green, blue-green and cryptomonad algal symbionts in the tissues have been reported in most groups of lower animals (Buchner, 1930, 1953).

The entire chronology presented here is summarized in Table 1.

In order to document this theory, the following, at the very least, are required: (1), the theory must be consistent with the geological and fossil records; (2), each of the three cytoplasmic organelles (the mitochondria, the (9+2) homologues, and the plastids) must demonstrate general features characteristic of cells originating in hosts as symbionts. None may have features that conflict with such an origin; (3), predictions based on this account of the origin of eukaryotes must be verified. The rest of this paper discusses the evidence for this theory in terms of assumptions based on molecular biology which can be made concerning evolutionary mechanisms.

3. Evidence from the Literature

3.1. ON CRITERIA FOR A NATURAL MICROBIAL PHYLOGENY

Throughout the systematic literature there exists the underlying assumption that the more traits two organisms possess in common, the more closely related they are. Higher organisms, such as vertebrates and flowering plants, show consistent morphological patterns which change in steps that can be related directly to adaptive value. Observation of living organisms, comparative physiology and anatomy, and the fossil record all provide much evidence for this assumption.

However, the lack of a comparable series of consistent morphological criteria has thwarted attempts to trace the evolutionary history of the "lower organisms", e.g. the "phycomycetes", the "mastigophora", the "sarkodina", etc., as any investigation of the literature on the subject will show.

On what, then, can the reconstruction of the evolutionary history of microbes be based? Recent advances in molecular biology provide some idea of the relative significance of various criteria in the development of microbial phylogeny, even if a great deal of fundamental data are still not available. For example, it is clear that we may *not* simply collect an arbitrarily large number of equal valued "traits" and try to group microorganisms on the basis of the largest number of such common "traits". Instead, as the genetic basis of many "traits" becomes known, we can rank them on the basis of the total number of single step mutations required to evolve them. In determining the relationship of two microbes—that is, the amount of time elapsed since they diverged from a common ancestor—we may ask: how many homologous base pair sequences in DNA do they share? The number of mutational steps which occurred to produce one from the other is related to the number of generations elapsed since the two populations diverged.

Thus, although two organisms may differ in a single measurable "trait", it must be clearly recognized that the genetic basis of such a trait may vary from a single (or a very few) DNA base pair changes to thousands or tens of thousands of such changes.†

Clearly, to construct a taxonomy reflecting natural phylogeny and not evolutionary analogy, microbes must be grouped on the basis of the total amount of genetic homology they share. A single mutation in a microorganism, resulting in a small chemical change with a profound phenotypic effect with respect to selection could easily mislead the taxonomist of lower organisms. On the other hand, organisms may share phenotypic traits and still be distantly related. For example, the fact that two microbes both metabolize glucose, but along entirely different pathways, implies a large number of different cistrons and, therefore, a long time since the two organisms diverged from a common ancestor.

Because of the lack of information, it is impossible of course to determine the number and order of DNA base pairs coding for a particular advantageous cistron, however certain criteria for ascertaining the degree of relationship between two microbes can be ranked in order of general validity (Table 2). For example, the homology of an entire metabolic pathway is a much more significant taxonomic criterion than the presence or absence of a single enzyme or pigment. In fact, the point at which metabolic pathways diverge in two otherwise similar microbes may help determine the elapsed time since the two microbes themselves diverged from a common ancestor.

The necessity for comprehending entire metabolic patterns, rather than individual biochemical traits, is especially relevant in botanical evolutionary

† This can be illustrated by an example drawn from two species of single-celled algae. Streptomycin resistant and streptomycin sensitive *Chlamydomonas* may differ only in a single trait—so may "bleached" and green strains of *Euglena gracilis* differ only in the single trait of "green color". However, it is likely that the difference in *Chlamydomonas* is due only to a single muton (Sager & Tsubo, 1961), whereas in clones of *Euglena* the one trait of green color (e.g. chlorophyll) in permanently "bleached" cells has been related to the presence of the entire chloroplast, and the potentiality for its formation (Lyman, Epstein & Schiff, 1961). Thus "green" and "bleached" *Euglena*, differing in the single obvious trait of color, betray a genetic difference of thousands of mutons. In the *Euglena* chloroplast there are at least 15 different kinds of enzymes (Smillie, 1963) and each one can roughly be estimated to be about 100 amino acid residues long. With a coding ratio of three nucleotides to each amino acid, the presence of a chloroplast implies enough genetic material, for structural genes alone, to code for $15 \times 100 \times 3 = 4500$ independent mutons. On the assumption that one cell in about a million contains a random mutation which turns out to be favorable for the evolution of the structural genes in the chloroplast, it would have taken about ($2^n \approx 10^6$, $n \approx 20$) twenty generations of *Euglena* to derive each of the 4500 favorable mutations in the pathway. Therefore, the trait of green color in *Euglena*, which may be permanently lost by exposure of the cells for a few minutes to ultraviolet light (Lyman, Epstein & Schiff, 1961), must have taken (very roughly but probably an extreme lower limit), 20×4500, or 90,000 generations to evolve.

TABLE 2

Taxonomic criteria in the formation of a natural phylogeny for microbes (listed roughly in order of relative importance)

Criterion	Techniques by which measured
Total homology of DNA base pairs	Direct DNA nucleotide sequence data Agar-gel technique for DNA homologies Ability to genetically recombine (i.e. classical genetic techniques) DNA base ratios on CsCl density gradient DNA denaturation (melting point) determinations
Homologous metabolic pathways	Classical biochemistry
Homologous cistrons, same "genetic code letters"	Homologous messenger RNA's (DNA-RNA homologies). Identity of individual transfer RNA's for specific amino acids
Ultrastructural morphology	Electron microscopy
Morphology and life cycle	Light microscopy, classical cytology
Single biochemical pigments, enzymes, etc., in common	Spectroscopy, classical biochemistry
Molecular structure of single pigment, or enzyme	Classical chemistry
Common phenotypic traits	Ability to grow on same carbohydrate, production of same end product, motility, etc.

work on "lower plants", where common photosynthetic pigments and metabolic products have been considered primary phylogenetic markers. Some groups of organisms have been considered closely related on the basis of their pigments [i.e. *Schizogoniacea, Euglenids, Conjugacea,* and other chlorophytes (Fritsch, 1935, p. 70), *Myxochrysis* and *Crysothylakion* (Copeland, 1956, p. 63]. Even red algae are thought to have been derived from blue-greens in some botanical literature:

"Fucoxanthin, which is shared by chrysomonads, diatoms and brown algae supports a common evolutionary origin for these members of the 'brown' line of metaprotists. The common possession of diadinoxanthin by diatoms and dinoflagellates is suggestive of an affinity between these groups. . . . The common possession of this class of chromoproteins—the bili-proteins—by the blue-green and red algae fits very well with derivation of the red algae photosynthetic apparatus from that of the blue-green algae. . ." (Dougherty & Allen, 1960, p. 129).

Classically photosynthetic organisms have been segregated into separate Kingdoms (or Classes) from their plastid-lacking counterparts, regardless

of their cellular morphologies which imply (at least to zoologists) that they should be grouped together [e.g. "*Chrysamoebida* (order): Rhizochrysidaeae, Chrysarachiaceae, Myxochrysidacea (families) . . . organisms in which only plastids distinguish them from various different groups of Rhizopods, Heliozoans and Sarkodina"] (Copeland, 1956, p. 63). Since zoologists assign relatively less importance to plastid characteristics, and tend to place such pigmented organisms in their respective protozoan groups, inconsistencies are rampant in the taxonomic literature of lower eukaryotes.

3.2. SOLUTION OF SOME TAXONOMIC PROBLEMS

According to the fundamental thesis of this paper, these problems are resolved if the validity of both views are seen simultaneously. For example, in the *Chrysamoebida*, it is unnecessary for zoologists to hypothesize that chrysophysean-type photosynthesis evolved separately, yet exactly analogously, in several diverse heliozoan, rhizopodal and sarkodinal lines; it is equally unnecessary for botanists to believe that the various rhizopods, heliozoans, and sarkodinae evolved from immediate chrysophysean ancestors by loss of photosynthetic plastids. Consistent with the above-mentioned hypothesis is the theory that algae, with chrysophysean photosynthetic characteristics, evolved millions of years before from photosynthetic prokaryotes and were acquired symbiotically in heliozoan, rhizopodal, and sarkodinal species, and that these prokaryotic algae became the obligately symbiotic plastids in the various protozoans. In other cases (e.g. *Chloramoeba* and *Chrysamoebae*), both chrysophysean and typical "green" prokaryotic algae were ingested by very similar amoeboflagellates whose descendants subsequently evolved "algal" ways of life. Presumably, too, typical green prokaryotic algae were acquired by euglenids, chloromonads, *Schizogoniacea*, etc., explaining the remarkable similarity in the chloroplast-related features in these different "host" organisms. There is really no other reasonable explanation for the evolution of certain natural groups of highly specialized flagellates which are found to contain distinct types of plastids with distinct pigments and storage products [e.g. *Cryptomonadina* (family): *Rhodomonas* (red), *Cryptochrysis* (chrysophysean), *Chilomonas* (colorless)]. As is now recognized in lichen classification, the host and plastid components of photosynthetic eukaryotes, like the component fungi and algae of lichens, ought to be classified separately. Perhaps, then, a natural phylogeny of the lower eukaryotes could be developed which is satisfactory to botanists, zoologists and mycologists.

In some cases, no doubt, the secondary loss of plastids from various protozoans have resulted in their return to the heterotrophic habit. The probability that the host can tolerate the loss of the symbiont must be

related to the extent to which the symbiosis itself has evolved. For example, *Paramecium bursaria* and various other animal cells harboring symbiotic algae can survive induced loss of their photosynthetic capabilities (Siegel & Karakashian, 1959). *Euglena gracilis* can survive loss of its chloroplasts. In both of these organisms, "curing the cells" of their photosynthetic algae or chloroplasts involves some loss of viability in most media, however (Karakashian, 1963). For example, *Paramecium bursaria* grown in the dark will lose its algae. Although this ciliate can still divide asexually, it cannot go through the meiotic events prior to conjugation in the absence of its symbiotic zoochlorellae (Siegel, personal communication). The symbiont-host relationship can be restored readily in *P. bursaria* by feeding the specific zoochorellae (which can be grown *in vitro*) to the "cured" ciliate (Siegel, 1960). However, growth of the chloroplast *in vitro* and its reintroduction into *Euglena* have posed insurmountable technical problems to date. The dependence of the "host" on the symbiotic plastid is, of course, still more pronounced in most higher plants unable to survive a loss of photosynthetic capacity at all except, perhaps, under very special conditions. (For example, people may keep albino corn alive by feeding the plants sugar directly through their leaves).

Thus, in general, a greater mutual dependency probably reflects a longer host-symbiont association. This is because new syntheses, made possible by the symbiosis, will be selected for and gradually will become necessary for survival. It is highly probable that many of the pathways particular to plant metabolism—the formation of the plant cell wall, alkaloid syntheses, formation of certain storage products, etc.—originate from the ancient protozoan-prokaryotic algal symbioses, analogous to starch synthesis in *Peliaina.*

"*Peliaina cyanea* is a flagellate (chrysomonad or cryptomonad in its affinities) which harbors from one larger to six smaller cyanella (blue-green algae). Rare assymetric fissions yield colorless monads (host flagellates) which produce oily reserves rather than starch like the *Peliaina* complex. Since free cyanophytes do not produce starch either, the complex has achieved a new function. The syncyanom must be of some antiquity as there is no way the flagellate could have ingested the cyanella. Phagotrophic, amoeboid phases have been described for some primitive flagellates, and the ingestion of a free living cyanophyte by an amoeboid ancestor presumably initiated the symbiosis . . ." (Lederberg, 1952).

On the basis of a very general biological argument too, it can be said that photosynthesis evolved in the prokaryotes and mitosis evolved in the protozoans. That is, from our knowledge of evolution in higher organisms, it seems that to identify the population in which a multicistronically determined trait evolved, related organisms must demonstrate a large range of small

variations in the given trait that can be correlated to specific selective environmental factors.

Lack of variation in a multicistronically determined trait in a natural group of organisms suggests the trait evolved earlier in some other ancestral population. (For example, in mammals: the metabolism of glucose; the histology of bone tissue; lungs developed from an outpocketing in the gut; the closed circulatory system containing red blood corpuscles; the vertebral column; the dorsal, hollow nervous system, etc.; in angiosperms: Krebs cycle intermediates; green plant photosynthesis; meiosis and fertilization; vascular tissue; seeds; etc.)

On the other hand, that the variations can be understood in terms of adaptive value to the population is considered evidence that, for example, these traits evolved in the following natural groups: triploblastic development in metazoans; the five-digit tetrapod in amphibious vertebrates; development through the total life cycle in dry environments in amphibians and reptiles; wide variety of beaks and feet forms in Galapagos Island finches; the mammary glands in mammals; and the flower in angiosperms.

We have already discussed some of the variations on the theme of mitosis in protozoans, sections 2.4 to 2.5.6; for the discussion of the variations in photosynthetic metabolism in contemporary prokaryotes, see sections 3.4 and 3.5.

3.3. GENERAL PROPERTIES OF SYMBIOSIS

The argument presented for the origin of the eukaryotic cell unequivocally points to the acquisition by symbiosis of mitochondria; the genome of the (9+2) complex flagellum; and the plastid. What are the general criteria of organelles derived by symbioses?

(1) A symbiont originated as a free-living cell and therefore must have once been able to replicate its own DNA on its own protein synthesizing machinery. As outlined above, these subcellular organelles are hypothesized to have evolved along the main line of terrestrial cellular evolution and, therefore, must have contained DNA, messenger and ribosomal RNA, etc., that is, all minimal requirements for cell reproduction common to terrestrial cellular life. This is not meant to preclude the possibility that alternative modes of cellular replication antedated the ones with which we are concerned (Pirie, 1959). Thus at the very least, a symbiont must have had: (a) DNA; (b) messenger RNA (mRNA) complimentary to that DNA; (c) a functioning protein synthesizing system; (d) a source of ATP and other nucleotides; (e) a source of small molecules from which to make proteins and nucleic acids; and (f) a cell membrane synthesizing system. Upon entry into a host, such a symbiont may lose from none to all of its

synthetic capabilities *except the ability to replicate its own DNA* and synthesize complementary mRNA from that DNA—the *sine qua non* of any organism. Hence, if any of these organelles originated as symbionts, their characteristic specific DNA must be present in the host at every stage of the host life cycle. It, of course, follows, too, that the ratio of host genome DNA synthesis to symbiont genome DNA synthesis must approximate 1 : 1. If the ratio is greater than 1 : 1 the host will outgrow the symbiont and give rise to daughters which lack it; if the ratio of host DNA synthesis to symbiont DNA synthesis is less than 1 : 1 the symbiont will outgrow the host and lysis will ensue.

By analogy with parasitism and mutualism in higher organisms, it is highly likely that after long association the redundancy intrinsic in symbiotic relationships will be selected against; the symbiont will tend to relegate all dispensable metabolic functions to the host. This tendency results ultimately in symbioses which become progressively more obligate.

(2) If an organism or organelle has been acquired by symbiosis, it will be retained intracellularly in its host if, and only if, there exists some mechanism to insure that each daughter cell of the host receives at least one copy of the symbiont genome at each division. Any mutation that insures the symbiont's distribution to both daughters in each division will be of high selective value for the complex (e.g. a common effective but expensive mechanism would be the presence of many copies of the symbiont within the host cells, increasing the probability that each daughter receives at least one symbiont). Indeed, when viewed in this way, many classical cytological observations on the behavior of centrioles, mitochondria and chloroplasts in cells of their "hosts" can easily be interpreted, as they indeed were, to be mechanisms that insure the genetic continuity of the organelle genome.

(3) If a cellular organelle is acquired by symbiosis, there should be no organism containing intermediate intracellular stages of the organelle. The entire series of metabolic capabilities conferred on the host by the symbiont must be acquired together, i.e. "packeted" as a unit.

(4) If the symbiont is lost, all metabolic characteristics coded for on the symbiont genome must be lost together. Once lost, a symbiont can never be regained unless it is reacquired by ingestion. Indeed, unless the reingestion quickly succeeds the loss, it is unlikely that precisely the same symbiont will be reacquired. For example, such reingestion has been suggested for the origin of *Glaucocystis nostoc*, an organism with the "host" features of an *Oocystaceae* containing distinctly blue-green algae-like, rather than "green", plastids (Fritsch, 1935, p. 186) and for *Gloeochaete*, "long referred as an anomalous genus of the *Myxophyceae*, but now known to represent a

colorless Tetraspraceous form in which the blue-green chromatophores are symbiotic blue-green algae" (Fritsch, 1935, p. 125).

Of course, a clear distinction must be made between the loss and the dedifferentiation of the symbiont. In dedifferentiation, the genetic potential for symbiont formation is retained implying a complete retention of the permanent symbiont genome and, therefore, of its DNA. Loss of the symbiont implies permanent loss of symbiont DNA.

Metabolic pathways reflected in some morphology (e.g. ribosomes, lysosomes, endoplasmic reticulum, nuclear membranes, etc.) which are coded for on the nuclear genome if lost from the cytoplasm, may be replaceable in the proper environment by the action of the nuclear genes. This can never be true of organelles which originated by symbiosis.

(5) Since any intracellular symbiont must have its own genes, a correlation can be made between genetic traits conferred on the host by the symbiont and the morphological presence of the symbiont. For example, in all eukaryotes the number of mitochondria and plastids will not necessarily be halved at meiosis and doubled at fertilization to establish their constancy. Hence, they will not necessarily display the Mendelian distribution of characteristics from generation to generation. The observations that in some cases mitochondria and plastids are inherited only with the larger female gamete in anisogametic fertilizations, led to the early hypotheses that these organelles were bearers of "cytoplasmic heredity". Thus, non-mendelian genetics should be found in those organisms in which mitochondria or chloroplasts are inherited uniparentally. The transmission of the trait should be associated with the donor parent [e.g. sea urchins where all paternal mitochondria are found in only one blastomere at the 32-cell stage (Wilson, 1925, p. 713). In exceptional cases this might apply to (9+2) homologues as well (e.g. patrilinear inheritance of the centrosome, e.g. in *Culex* (Darlington, 1958, p. 174)]. In general, of course, genetic traits carried by the (9+2) chromosomal centromeres will show strict Mendelian inheritance patterns.

(6) If an organelle originated as a free-living cell, it is possible that naturally occurring counterparts still can be found among extant organisms. Even if precise extant morphological and physiological codescendants cannot be found, the organelle must have genetic and physiological characteristics known to be consistent with those generally present in terrestrial cells.

Applying the above criteria, the nucleus of the eukaryotic cell could not have originated by symbiosis. The great body of literature on the genetics and cytology of higher organisms defends the thesis that nuclear genes control cytoplasmic syntheses which make possible the duplication of

nuclear DNA and growth of the cell prior to the next division (Brachet, 1957). Recent evidence indicates a large number of sites spread over many nuclear chromosomes control the production of the cytoplasmic RNA (Prescott, 1964). The nucleus and cytoplasm of the eukaryotic system, are clearly part of a highly integrated continuous system; it seems there is little evidence that can be cited for their independent origins.

To document this theory, the rest of the discussion is devoted to the origin of prokaryotic cells hypothetically destined to become organelles of eukaryotes, and to the present status of cytoplasmic organelles in terms of the general criteria for symbiotic origin developed above.

3.4. ANCIENT ANAEROBIOSIS AND MICROBIAL PHOTOSYNTHESIS

The earth's atmosphere, both in its absence of hydrogen and presence of large quantities of oxygen is cosmically atypical. Although there is no consensus on details astronomers and geologists today believe that the original terrestrial atmosphere, which was subsequently lost, was composed primarily of hydrogen. Many different laboratory attempts to simulate the origin of life have been made; a variety of molecules (e.g. amino acids, pyrimidines, ATP) found in contemporary organisms can be produced if the net chemical conditions are reducing. However, if conditions are oxidizing, such as they are in the present atmosphere, the production of common organic compounds is exceedingly inefficient. All of the evidence is consistent with the widely accepted hypothesis that life arose under reducing conditions of the primitive atmosphere (Sagan, 1965*b*).

Photosynthesis, in the general sense of the utilization of solar energy in the production of organic compounds, probably antedates the origin of life itself. The strong ultraviolet light absorption capabilities of the major nucleic acid components, of ATP, and of amino acids and peptides may have led to their local accumulation in the absence of replicating cellular systems upon which natural selection acted, as early as 4 to 5 billion years ago (Sagan, 1961).

When did the atmosphere become oxidizing? Geochemical studies independent of biological speculation provide evidence on the question of dating the transition to the oxidizing atmosphere. As recently as 2 to 3 billion years ago, sediments in Canadian, Brazilian and South African shields were deposited containing uraninite (UO_2) that had not been oxidized to pitchblends, suggesting the absence of an appreciable oxygen partial pressure at the time of deposition. However, oxidized rocks as old as $2{\cdot}5 \times 10^9$ years have been found, suggesting that free oxygen was abundant enough to oxidize iron to form limonite red-beds at this time (Rutton, 1962).

Recently, Cloud has reviewed this literature and amassed a good deal of evidence resolving the apparent discrepancies in the data. He has found microstructures, indisputably fossils, which he considers to greatly resemble extant prokaryotes (blue-green algae and iron bacteria) in rocks definitely dating $2{\cdot}1 \times 10^9$ years ago. [In fact, microstructures of fossil prokaryotes—(e.g. *Eobacterium isolatum*) have now been reported in rocks as old as $3{\cdot}1 \times 10^9$ years (Barghoorn & Schopf, 1966).] In concluding his review, Cloud points out,

"The combined evidence of paleontology and stratigraphy, therefore, indicates that the potentiality for the evolution of oxygen by green plant photosynthesis existed at least $2{\cdot}7$ to $2{\cdot}1 \times 10^9$ years ago, and that atmospheric oxygen first began to be available in relatively large quantities probably about $1{\cdot}2 \times 10^9$ years ago (a conclusion independently reached on a different line of reasoning by Lepp and Goldich)" (Cloud, 1965).

(The "green plant" photosynthesis referred to by Cloud includes prokaryotic blue-green algal photosynthesis; it is distinguished from bacterial photosynthesis by the elimination of gaseous oxygen in the process.)

Correct identification of the fossils implies the origin of photosynthetic metabolism which uses the visible portions of the spectrum, occurred in cellular systems earlier than $2{\cdot}1$ billion years ago or even $3{\cdot}1 \times 10^9$ years ago. This is consistent with independent astronomical arguments which indicates that uv absorbing reduced compounds were probably in the atmosphere contemporaneous with these prokaryotic photosynthetic algae (Sagan, 1965*b*).

In any case, the utilization of visible light in microbial photosynthesis must have evolved more than $1{\cdot}2 \times 10^9$ years ago (the time at which free gaseous oxygen was clearly present) because it is likely that, at that time, the ultraviolet light that penetrated the ozone layer of the upper atmosphere was insufficient to provide energy for the formation of organic compounds upon which all life is based. Even if the production of new organic matter continued, it would have been destroyed by oxidation. Thus, within the confines of these dates, this outline for the evolution of cellular photosynthesis was reconstructed.

Porphyrins (metal-chelated tetrapyrroles such as those found in catalase and peroxidase) of some kind are of universal occurrence in all extant cells with the exception of some obligate anaerobes (Lascelles, 1964). Their ubiquity has been related to their ability to reduce mutagenic oxidizing agents. Molecular oxygen is continually being produced by the photolysis of water in the upper atmosphere and the escape of hydrogen (Urey, 1959). This oxygen must have been lethal to the early self-replicating systems. Atmospheric photoproduction of oxygen throughout the ages presumably provided selection pressures for

the retention of these ancient cistrons involved in the synthesis of porphyrin antimutagens. By hypothesis, the incidental fact that these antimutagenic tetrapyrole compounds were strong visible light absorbers was later put to advantage in the evolution of chlorophyll-mediated microbial photosynthesis (Sagan, 1961).

The CO_2 fixation (the dark reactions) of photosynthesis is known to be present for the production of cell material in many different non-photosynthetic organisms (e.g. chemolithotrophs: bacteria capable of oxidizing hydrogen gas, sulfide, sulfur, ammonia and nitrite). It is possible that mutations causing the porphyrins to be used in cellular photoproduction of ATP originally occurred in microbes which already contained the CO_2 fixing "dark reactions". This could have resulted in the evolution of the fundamental photosynthetic mechanism, providing a new source of ATP, produced by visible light absorbed by porphyrins. In some organisms, chemotrophic or direct heterotrophic methods of obtaining ATP presumably were then replaced by the visible light-chlorophyll mediated methods characteristic of all microbial photosynthesizers.

The relationship of porphyrin synthesis to intermediary metabolism, i.e. the synthesis *via* glutamate, α-ketoglutaric acid, and succinyl-CoA, suggests that the porphyrins originally selected for because of their antimutagenic properties—for example, in the reduction of hydrogen peroxide—may have eventually been used in the more efficient oxidation of carbohydrate. Organisms unable to form these porphyrins were doomed to eternal obligate anaerobiosis (Stanier *et al.*, 1963, p. 85). Thus, heterotrophic, anaerobic respirers (microbes which oxidize carbohydrates in the absence of molecular oxygen to make ATP *via* iron-chelated porphyrins such as cytochromes) could also have evolved from primitive porphyrin synthesizers.

Eventually in prokaryotic anaerobic photosynthesizers, mutations must have occurred that led to the development of organisms capable of using H atoms from (the much more abundant) water instead of from (the less abundant) hydrogen gas or hydrogen sulfide (e.g. the anaerobic photosynthesizer, *Thiorhodacea*) as electron donors to chlorophyll. The natural consequence of these mutations was the elimination of oxygen derived from that water in the photosynthetic process.

The fact that oxidized limonite red-beds are as old as $2{\cdot}5 \times 10^9$ years is strong evidence that mutations for oxygen eliminating photosynthesis occurred in some microbial autotrophs well before that time.

3.5. ATMOSPHERE OXYGEN AND THE ORIGIN OF AEROBES

The presence of increasing amounts of oxygen, produced by the new photosynthesis must have been extremely deleterious: there was no longer

any abiogenic synthesis of organic compounds; the obligate anaerobes found progressively fewer niches; and all life became ultimately dependent on visible light photosynthesis to produce nucleotides and oxidizable carbohydrates. Under such conditions, constant selection pressure must have been placed on any microbes capable of tolerating and utilizing oxygen. In the case of the cytochrome-containing anaerobic respirers—cells which already had genes to code for earlier steps in the respiratory pathways—mutants were selected that could transfer hydrogen atoms to oxygen (instead of to nitrogen or sulfate). This could have resulted in the evolution of ATP pathways involving the complete oxidation of production *via* the aerobic carbohydrate. Such mutations leading to the aerobic breakdown of carbohydrates presumably occurred in some photoautotrophs too, evolving the ancestors of blue-green algae.

It is consistent with both microbial metabolism and the geological evidence to assume, then, from 2·1 to $0{\cdot}6 \times 10^9$ years ago the prokaryotic photosynthesizers eliminated more and more oxygen into the atmosphere. In response to this, different types of microbes evolved (including the proto-mitochondrion and prokaryotic aerobic algae), capable of coping with the increasing abundance of oxygen. The goal of the long metabolic sequences coded for on microbial genomes was then, as it is today, maintenance of the highly reduced surroundings required for reproduction. Ironically, the change from reducing to oxidizing conditions to which microbial cells were forced to adapt had been caused by the cells themselves.

Interestingly enough for our argument, although micro-fossil forms quite analogous to extant prokaryotes are found from $3{\cdot}1 \times 10^9$ years ago, no eukaryotic algae appear in dated rocks until the dawn of the Paleozoic. *Dasycladaceans* are known from early Ordovician, about $0{\cdot}4 \times 10^9$ years ago, and algal filaments perhaps reds or brown (*Epiphyton*) are known as old as early Cambrian 0·50 to $0{\cdot}55 \times 10^9$ years ago (Cloud, 1965, personal communication).† Thus, not only are "missing links" between prokaryotic and eukaryotic algae conspicuously absent from all present day flora, but they are missing from the fossil record during those 2700 million years (i.e. from $3{\cdot}1 \times 10^9$ to $0{\cdot}4 \times 10^9$ years ago), in which first prokaryotic cells (Schopf & Barghoorn, personal communication) and later atmospheric oxygen were known to be present!

Besides the direct evidence of the fossil record, there are biological reasons for regarding photosynthesis as a fundamentally anaerobic process which evolved in prokaryotes. Both blue-green algae and bacterial photosynthesizers are clearly related to other prokaryotes.

† *Note added in proof:* It is now likely that Australian (Bitter Springs) PreCambrian fossils about $0{\cdot}8 \times 10^9$ years old contain primitive eukaryotic coccoid algae (Schopf & Barghoorn, 1966, personal communication).

All bacterial photosynthesizers are morphologically typical gram negative bacteria. The exception to this is *Rhodomicrobium*, photosynthetic budding bacteria, which also have typically prokaryotic nonphotosynthetic counterparts (*Hyphomicrobium*). In all these bacterial photosynthesizers, the photosynthetic process itself is anaerobic. It is only in the dark that any bacterial photosynthesizers are aerobic and respire. Even in blue-green algae, oxygen uptake is inhibited by light (Marsh, Galmiche & Gibbs, 1964).

Blue-green algae also have nonphotosynthetic morphological counterparts among prokaryotes (the filamentous gliding bacteria). Some of these, like *Beggiota*, metabolize H_2S to form sulfur. These organisms grow using CO_2 as their only source of carbon; the H atoms from H_2S are transferred to "fix" CO_2 for reduction to cell material. In the blue-greens H atoms from H_2O are transferred to "fix" CO_2 for reduction to cell material using photosynthetically produced ATP. Thus these gliding bacteria are physiologically as well as morphologically related to blue-greens.

The fact that some blue-greens metabolize along the pentose phosphate (rather than the Embden-Meyerhof pathway so ubiquitous in mitochondria-containing eukaryotes) also reflects their origin and relationship to prokaryotes (Fewson, Al-Hafidh & Gibbs, 1962). This difference in the first steps of carbohydrate metabolism in these two great groups of oxygen eliminating algae indicates their ancient evolutionary divergence† (Haldane, 1954).

The monophyletic evolution of eukaryotic algae from blue-greens is hard to reconcile with many other facts. Blue-greens are typical prokaryotes: they lack a membrane bounded nucleus, mitochondria, flagella, mitosis, sex, simultaneity of respiration and photosynthesis, and, in general, they are smaller than other algae. Furthermore, no intermediate organisms between blue-green and green algae have ever been found, even though they have been extensively sought.

For a comprehensive review of recent literature demonstrating the striking similarities in cell structure of the blue-green algae and the bacteria, see Echlin & Morris, 1965.

Why then are the photosynthetic mechanisms in prokaryotic blue-green algae and eukaryotic green algae so similar? If the prokaryotic blue-greens were not ancestral to the eukaryotic algae and the higher plants, what organisms were?

† This type of reasoning was first used by Haldane in 1929. He pointed to the uniformity of the first steps in anaerobic metabolism and the diversity in the molecular oxygen utilizing —or avoiding—later steps to indicate the greater age of the anaerobic processes. From this, he inferred something of the nature of the reducing atmosphere—in the absence of the laboratory simulation experiments and the geological data that is available to us today!

This apparent paradox can be resolved by recognizing the validity of the thesis presented in this paper, namely, that the evolution of photosynthesis preceded the evolution of the eukaryotic cell by millions of years, and that green plant oxygen-eliminating photosynthesis—so characteristic of both blue-green algae and "chlorophytes"—evolved in prokaryotes and was later acquired symbiotically by various eukaryotes.

This view is valid only if the evolution of the eukaryotic cell itself from an aerobic, heterotrophic ancestor can be understood. What evidence have we that mitosing eukaryotic cells evolved monophyletically and independent of the evolution of photosynthesis? What is the basis for the claim that mitochondria and (9+2) homologues originated as endosymbionts, and that our hypothesis concerning the evolution of the eukaryotic cell is, at least in essential outline, correct?

3.6. THE REPRODUCING MITOCHONDRION

A plethora of recent studies elegantly reviewed by Gibor & Granick have presented inexorable testimony for the following: mitochondria contain specific DNA and RNA; they are self-duplicating bodies that do not arise *de novo*; the multigenic system of the organelle is responsible, in part, for the specific biochemical properties of the organelle; and mitochondrial development (in yeast cells, at least) are controlled by an adaptive mechanism which is responsive to oxygen. There have been some reports that mitochondria are capable of limited incorporation of amino acids into proteins and they contain their own protein synthesizing mechanisms, as well. For a detailed description and bibliography of the pertinent literature, the reader is referred to this excellent review (Gibor & Granick, 1964). That mitochondria have sources of ATP and small molecules, is indisputable. Whether the mitochondrial membranes are coded for by the mitochondrial genes themselves is not clear. In any case, the mitochondria satisfy the first criteria for cells originating as endosymbionts as discussed earlier (section 3.3).

In 1927, Wallin argued that mitochondria originated as endosymbionts in higher cells. His evidence was based on the size, shape, staining properties and general cytological behavior of the organelles which he claimed were comparable to bacteria. Of course, at that time he could have had little concept of their physiology. The most convincing evidence for the genetic autonomy of mitochondria involved studies of their continuity. These observations reviewed by Wilson in 1925, indicated that mitochondria are invariably included in sperm cells and are, in general, present at every stage of the life cycle in eukaryotic cells. Some of the mechanisms by which mitochondria are retained in daughters during cell divisions are listed in Table 3. Full discussions appear in the classical literature (Wilson, 1925).

TABLE 3

Cytological mechanisms for retention of organelles throughout life cycle

Organisms	Mechanism
A. *Mitochondria*	
Many plants and animal cells; dividing germ cells of vertebrates; cleavage stages	Many mitochondria randomly distributed throughout cell (Wilson, 1925, p. 712)
Spermatocytes of some scorpions, such as *Opisthancanthus*, *Hadrurus*, etc.	Primary spermatocytes: small and numerous mitochondria join together to form 24 spheroids Secondary spermatocytes: 12 spheroids are segregated to each daughter cell. Spermatids: six spheroids are segregated to each pole; thus six mitochondrial spheroids are present in each sperm cell (Wilson, 1925, p. 163)
Spermatocytes of scorpion, such as *Centrurus*	Primary spermatocytes: mitochondria aggregate into ring-shaped body, oriented on spindle, is cut transversely by division of cell into two half rings; each half ring forms a rod. Secondary spermocyte: each rod is carried on spindle and cut by cell division into a half rod (Wilson, 1925, p. 365)
Spermatocytes of worm such as *Ascaris*	Mitochondria are oriented on spindles toward centrioles. They do not divide but are segregated to daughter cells by virtue of their orientation (Wilson, 1925, p. 163)
Some insects, such as *Hydrometra*	Elongate rods of mitochondria are oriented on spindles; some of these mitochondrial rods are cut by cell equator (Wilson, 1925, p. 164)
Some ciliates	Mitochondrial divisions of numerous mitochondria synchronous with nuclear division (Wilson, 1925, p. 13)
Vicia, a bean	Two groups of mitochondria orient at opposite poles at division (Wilson, 1925, p. 163)
Micromonas, a photosynthetic flagellate	One mitochondrion divides synchronously with nucleus (Gibor & Granick, 1964)
B. *Chloroplasts*	
Diatoms	Small and constant number evenly distributed in mitosis (Darlington, 1958)
Micromonas, a photosynthetic flagellate	One chloroplast divides (Gibor & Granick, 1964) synchronously with nucleus
Chlamydomonas, a photosynthetic flagellate	One large chloroplast cut by cleavage plane (Gibor & Granick, 1964)
Most higher plants	Many chloroplasts randomly distributed at mitosis (Wilson, 1925, p. 162)

Recently, the absence of visible mitochondria in the cytoplasm of anaerobic yeast (as seen through the electron microscope) has again been taken as evidence for the lack of genetic continuity of the organelle. Since we now know the fundamental replication event takes place on the molecular level, conclusive experimental evidence for the contrary could come from simply showing that yeast "satellite band DNA" (e.g. mitochondrial DNA) is still present in those organisms which lack visible mitochondria but retain the potential to form them.

The entries in Table 3 can be interpreted as mechanisms which insure that each daughter cell of the host receives at least one copy of the symbiont genome at each division. In the formation of sperm, mitochondria are often wrapped around the axial fiber, a (9+2) homologue, insuring continuity of the organelle through fertilization as well (Wilson, 1925, p. 373) (criterion (2), section 3.3).

Do mitochondria fulfil other requirements for organelles originating in symbionts? There seem to be no extant examples of organisms that totally lack mitochondria and contain other characteristics of eukaryotes. This is probably because the mitochondrial symbiosis is so ancient it is obligate in all eukaryotes: total loss of the organelle is invariably lethal (criteria (3), section 3.3). However, neither are there nucleated or plastid-containing organisms which contain mitochondrial enzymes "unpackaged" (criteria (4), section 3.3).

Examples of mitochondrial cytoplasmic heredity are well known (Gibor & Granick, 1964; Jinks, 1964) (criterion (5), section 3.3). Many aerobic bacteria provide free-living extant counterparts to the protomitochondrion, since aerobic cytochrome-mediated glucose metabolism is well known among bacteria (criterion (6), section 3.3).

Regardless of the history, it must have taken thousands of mutational steps to evolve mitochondria (i.e. approximately 100 enzymes $\times$ 100 amino acids per enzyme $\times$ 3 nucleotide pairs per amino acid $\approx 3 \times 10^4$ nucleotide pairs). The evolution of the chloroplast in an aerobic cell containing mitochondria resulting in green plant photosynthesis precisely analogous to that of blue-green algae is highly improbable. Such a monophyletic origin of chloroplasts would require thousands of further highly specific mutations to evolve photosynthesis. The absence of both fossil evidence and extant intermediate organisms in the evolution of this "primitive phytoflagellate" makes it unlikely that plastid-contained aerobic photosynthesis itself evolved in a cell containing mitochondria. Since we have already presented evidence that photosynthesis is fundamentally an anaerobic process which evolved in prokaryotes (section 3.5), this is especially unlikely.

On the other hand, if the evolution of mitochondria followed the evolution of plastids, there is no obvious reason why photosynthetic eukaryotes

should show remarkable uniformity in mitochondrial structure and metabolism. Plastid-containing organisms with alternative carbohydrate metabolic patterns should have evolved, especially since such alternative pathways are known to be present in blue-green algae.

3.7. THE REPRODUCING (9+2) HOMOLOGUES AND THEIR RELATIONSHIP TO THE NUCLEUS

". . . that a fundamental dualism exists in the phenomenon of mitosis, the origin and transformation of the achromatic figure being in large measure independent of those occurring in the chromatic elements. Mitosis consists, in fact, of two closely correlated but separable series of events" (Wilson, 1925).

The homology of the "achromatic figure" (i.e. mitotic apparatus, basal bodies, centrioles, etc.) and its relative independence of nuclear chromatin is at the base of the above statement which summarizes the results of some 40 years of cytological observation, by perhaps the finest biologists of the time (*c.* 1885–1925).

Classical cytogenetics and more recent experimental work involving modern techniques have confirmed and extended the observations of the early workers. Especially relevant are the careful studies of Cleveland which experimentally disassociate chromosome replication and cell division.

"Oxygen concentrations of 70–80% destroy all the chromosomes of the hypermastigote flagellate, *Trichonympha*, provided the oxygen treatment was carried out during early stages of gametogenesis when chromosomes are in process of duplicating themselves. This treatment does no damage to the cytoplasm and its organelles, following the loss of chromosomes. The centrioles function in the production of the achromatic figure (e.g. the mitotic apparatus), the flagella and the parabasal bodies. Then the cytoplasm divides, thus producing two anucleatic gametes which make some progress in the cytoplasmic differentiation characteristic of the normal male and female gametes of *Trichonympha*" (Cleveland, 1956).

On the other hand, Cleveland's observations of binucleate 5-centriole cell showed that ". . . without centrioles no achromatic figure is formed, there is no poleward movement of the chromosomes to form daughter nuclei. The chromosomes reproduce themselves but the nucleus does not. However, two or more centrioles must be present and must be fairly close to the nucleus if the nucleus is to reproduce itself" (Cleveland, 1963). When there is a choice in these multicentrioled cells, chromosomes will move along other than the central spindle.

The fundamental difference between eukaryotic mitosis and the equal distribution of genes in prokaryotic cell division is the total amount of DNA

which can be distributed to the daughter cells. If newly synthesized cellular DNA could be attached to some intracellular self-replicating body which at the time of host division segregated from *its* sister offspring, then a mechanism for equal distribution of genetic material could result. This mechanism, operating quite independent of the "messages" carried by the host DNA, would simply insure the segregation of the newly synthesized host DNA associated with the self-replicating intracellular body. In such a scheme, it is unnecessary to explain how the self-replicating body differentiated from the nucleus *before* it was selected as a division center. The alternative assumption that a pre-existing replicating endosymbiont, the basal body of the flagellum, was utilized in a new role, is consistent with traditional belief—that evolution is opportunistic and not foresighted. The role of host chromatin segregation is the recognized function of the centromere; cells lacking centromeres simply do not arrive at the poles of the mitotic spindle to be incorporated into daughter nuclei, and chromosomes always proceed to the poles centromere first. Chromosomes may contain two centromeres that travel to opposite poles of the dividing cell; often when these dicentric chromosomes break, each fragment attached to its centromere is incorporated into the resulting daughter cell.

The function of the flagellar basal apparatus in mitosis and its use in distribution of cellular organelles was widely recognized in the classical cytological literature (Table 3). A striking example of this is to be found in *Trypanoplasma* (Belar, 1915). The division of the blepharoplast at the base of the flagellum—called the *blephoplasteilung*—is quite as conspicuous as the nuclear division, forming an apparent second "mitotic apparatus" related to the flagella [for example, Fig. 2. V (d); [criterion (2), section 3.3].

In *Leishmania* the kinetoplast attached to the flagellar apparatus, for example, is Fuelgen positive and known to divide. Very clear evidence for a specialized DNA satellite band associated with the organelle has been presented (Du Buy, Mattern & Riley, 1964) consistent with evidence that the organelle incorporates thymidine into DNA (Gibor & Granick, 1964). Electron microscopy indicates that the kinetoplast contains the single mitochondrion which differentiates during the part of the life cycle in which oxidative metabolism is required. It is possible that a specialized association between (9+2) homologue and the one mitochondrion has evolved to insure distribution of the mitochondrion to the daughter cells in a way analogous to the distribution of daughter nuclear genomes. The homology of the kinetoplast DNA may be ascertained by DNA hybridization experiments with the mitochondrial and flagellar DNA's.

The homology of the centriole and the flagellar basal body, first suggested in 1898 (Wilson, 1925, p. 697), is now widely accepted especially as their

structure (as revealed by the electron microscope) has been elucidated (Sleigh, 1962). The excellent evidence for the "genetic autonomy" of these cytoplasmic self-replicating (9+2) organelles has been well reviewed (Jinks, 1964). There are reports in the literature of irreversible losses of (9+2) homologues (Lederberg, 1952; Jinks, 1964) (criterion (4), section 3.3). A specific (9+2) homologue DNA which presumably codes for the characteristic proteins, has been suggested by some studies (Seaman, 1960) but has not been definitely shown (Hoffman, 1965) (criterion (1), section 3.3). No intermediate organisms between those containing the (9+2) complex flagella and the simple eubacterial flagella are known (criterion (3), section 3.3). Hence, the origin of the (9+2) homologues as endosymbionts is not inconsistent with the evidence; but the argument that these homologues were of exogenous origin and did not pinch off from the cell (i.e. did not originate as episomes) must be justified.

For various reasons, spirochaete or spirochaete-like organisms have been suggested as likely candidates for free-living counterparts of the motile (9+2) endosymbiont which later differentiated into the flagellum, centrioles, and chromosomal centromeres. Spirochaetes are known to be associated with protozoans; for example, in *Dienympha* and *Pyrsonympha* (eukaryotic animal flagellates found in insects) the family (Dienymphida) to which they belong has been characterized by the following description: ". . . elongate flagellates, the four or eight anterior flagella adherent to the body and spirally twisted with it, free at their distal ends. Often they are beset with spirochaetes which have been mistaken for additional flagella; the family has been misplaced in the order *Hypermastigina*" (Copeland, 1956, p. 166). The same is true of another family of eukaryotic flagellates, the *Devescovinida*: "Spirochaetes which share the habitat of these organisms are commonly found adhering to their cell membranes, and were mistaken for additional flagella in the original descriptions of some of the genera" (Copeland, 1956, p. 167).†

Spirochaetes are approximately the same size as flagella, they are made of subunit strands (Stanier *et al.*, 1963, p. 158) varying in number depending on the species. They are usually found in micro-aerophilic environments; they are always motile; their motility is sensitive to ATP; and, like flagella, they divide longitudinally. Indeed, it would be most interesting if the flagellar ATPase, other characteristic proteins (Gibbons, 1963), and (9+2) sub-

† *Note added in proof:* Recent Elegant studies of *myxotrixa paradoxa* indicate symbiotic spirochaetes on the surface of these cells are responsible for the hosts' movements! Moreover these flagellates typically have at least three different types of symbiotic prokaryotes associated with them. (Grimstone, A. V. & Cleveland, L. R. *Proc. R. Soc. Lon.* **159**, 668, 1964.)

structure could be identified with the proteins and axial fiber of some free-living spirochaete (criterion (6), section 3.3).

If the flagellar basal body had escaped from the host genome for the purpose of distributing nuclear DNA, it is likely that such episomal escape would have occurred in many different lines of microbes and that not all extant examples would be homologous. There is no immediate selective advantage to the escape of such a piece of host nucleic acid. If episomal in origin, we might expect examples of organelles which lyzed the host for their own continued replication. If (9+2) homologue centrioles pinched off and were selected for because they distributed cellular DNA efficiently, they should not necessarily be related to the flagella at all. If they escaped from the nucleus, these (9+2) homologues should be more sensitive to treatments which affect the nucleus than to those which are related to the destruction of the flagella. In fact, the opposite is true (Weinrich, 1954, p. 135; Yow, 1961).

3.8. THE REPRODUCING CHLOROPLAST

Presumably because it was the least ancient of the symbioses, the exogenous origin of the photosynthetic plastid is the easiest to defend (Ris & Plaut, 1962; Echlin, 1966). Chloroplast DNA has been found in many different photosynthetic eukaryotes. [In *Euglena*, direct autoradiographic evidence for nuclear and plastid DNA has recently been presented. The *in situ* DNA has been correlated with the presence of labeled main band (nuclear) and satellite band (chloroplast) DNA on a cesium chloride density gradient (Sagan, Ben-Shaul, Schiff & Epstein, 1965).] This satellite DNA band is totally absent in permanently "bleached" cells, those which permanently lack the potential for chloroplast formation (Leff, Mandel, Epstein & Schiff, 1963) (criterion (4), section 3.3). The disappearance of the "satellite band" DNA has been correlated with the ultraviolet treatment which permanently "cures" *Euglena* of its chloroplasts (Edelman, Epstein & Schiff, 1964; Edelman, Schiff & Epstein, 1965).

Other characteristics implied by an endosymbiotic origin have been reported for chloroplasts as well: chloroplast-specific RNA complimentary to chloroplast DNA (Eisenstadt & Braverman, 1963); ribosomes (Lyttleton, 1962); and chloroplast ribosomal RNA; evidence for DNA-dependent RNA synthesis; and the uptake of radioactive amino acids into isolated chloroplasts (Gibor & Granick, 1964).

In summing up both the classical observations on replication of chloroplasts and the recent work on the biochemistry of the organelle, Gibor and Granick provide convincing evidence that: the plastids (like the mito-

chondria) contain DNA and RNA; they are self-duplicating bodies which do not arise *de novo*; the DNA represents a multigenic hereditary system which is not derived from the nucleus and is, in part, responsible for the biochemical properties of the organelle; and that the differentiation of the mature chloroplast from the proplastid is an adaptive system responsive to visible light (criterion (1), section 3.3).

Some mechanisms which assure distribution of at least one copy of the plastid to each daughter of the host throughout the life cycle in plants are summarized in Table 3 (criterion (2), section 3.3).

Examples of organisms which lack plastids but are clearly counterpart to plastid-containing cells are very well known (e.g. *Astasia*, *Polytoma*, etc.). Blue-green algae themselves may be considered free-living prokaryote counterparts of plastids (criterion (6), section 3.3). Despite the search for them, neither fossil nor extant examples of intermediate organisms between the plastid-lacking blue-greens and the plastid-containing eukaryotes have ever been found (criterion (3), section 3.3).

"Cytoplasmic heredity" was first discovered in cases of uniparental chloroplast inheritance. The literature has been well reviewed (Jinks, 1964; Granick, 1962) (criterion (5), section 3.3). Characterization of the phenomena known collectively as "cytoplasmic heredity" is based on the non-chromosomal (and therefore non-mendelian) inheritance of genes. The logical explanation for cytoplasmic non-mendelian genetic systems is based on their legacy as genes of once free-living cells which have become organelles, i.e. hereditary endosymbiosis (Lederberg, 1952).

Apparently another major piece of circumstantial evidence for the endosymbiotic origin of these organelles is their size. We now know that replication takes place strictly on the molecular level; the flagellum itself is the size (and indeed the shape) of a respectable prokaryote, as are the mitochondria and plastids. The self-replication of such organelles of course involves synthesis of all the non-nucleic acid components for perpetuation. The probability that bodies of such enormous size (from a molecular point of view) would form multigenic systems in the cytoplasm is extremely low. The explanation that ". . . certain mutations could occur independently in each organellar DNA unit, and these mutations could be carried along so that, when drastic environmental changes occurred, there could be selection for the most suitable organelles. Thus, a multiple number of mutated organelles per cell could provide for more rapid evolutionary change" (Gibor & Granick, 1964) is patently Lamarckian. These were, however, the reasons given by Gibor & Granick for the large number of remarkably consistent experimental results implying genetic continuity of mitochondria and plastids.

4. Some Predictions

It is likely that the classifications presented in the phylogenetic tree (Fig. 1) err in that the author lacks first-hand knowledge of most of the organisms. It is also true that flagella and chloroplasts can be secondarily lost. However, if the theory presented in this paper is correct, all eukaryotes should ultimately be classified completely, correctly, and consistently according to their position in the origin of mitosis. Consistent with this view is the empirical fact that the flagellar attachment has proven to be a reliable taxonomic criterion.

Analogous to the quantitative relationship between DNA and ploidy, satellite DNA correlated specifically with the various organelles should be found in cells in direct proportion to the number of organelles in these cells. No eukaryotic cell having flagellar basal bodies, cilia, centrioles, centromeres, or any other of the homologues can lack (9+2) homologue-specific DNA. It is likely that this DNA has evaded detection because it has very little metabolic responsibility and needs to be only a few cistrons long to code for its few specific proteins. Identification of (9+2) homologue-specific DNA and a complete characterization of its RNA and of its limited biochemical functions should eventually be possible. This is true of mitochondrial and plastid DNA as well.

If these organelles did indeed originate as free-living microbes, our advancing technology should eventually allow us to supply all growth factors requisite for *in vivo* replication of all three of them—the *coup de grace* to genetic autonomy.

It is likely that biochemical pathways entirely unique to eukaryotes (e.g. steroid synthesis) should have component parts coded for by more than one organellar genome. [For example, it is possible that the biochemical pathway to squalene in steroid synthesis is under nuclear control, and that the subsequent members of the biosynthetic chain—those admittedly tightly bound to particulate elements in the cell—are under the control of the mitochondrial genome (Block, 1965).] The component parts of metabolic pathways in plant metabolism—analogous to starch synthesis in *Peliaina* (Lederberg, 1952) (section 3.2) for example—may eventually be understood in terms of "complementation", e.g. syntheses made possible by the presence in the cell of at least two genomes—host and plastid.

In keeping with the hypothesis, the following organisms should have evolved: a free-living complex flagellar counterpart; a free-living mitochondrion counterpart; and a heterotrophic prokaryote capable of ingesting cells. Free-living cells co-descendant with eukaryotic organelles might still contain cistrons homologous to those in (9+2) homologues, mitochondria, and plastids. For example, we may one day find different types of blue-green

algae that are co-descendant with typical chrysophsean and rhodophysean plastids, which contain DNA with cistrons homologous to those in the plastids.

If the theory is correct all eukaryotic cells must be seen as multi-genomed systems. This implies that a goal of cellular chemistry is understanding the way in which all biochemical reactions are coded off the nucleic acid of the nucleus and the subcellular organelles. All eukaryotes must contain at least three specific types of DNA: nuclear, mitochondrial, and (9+2) homologue. An additional DNA that is associated with the chloroplasts must be found in all eukaryotic plants. The plastids of organisms sharing similar photosynthetic metabolic characteristics (e.g. dinoflagellates, brown algae, and diatoms; red and blue-green algae) should have homologous plastid-specific nucleic acid. At the same time, those organisms which demonstrate "host homologies" (e.g. non-pigmented and pigmented dinoflagellates, *Schizogoniacea* and *Porphyra*) should share homologous nuclear, and not necessarily plastid, DNA's. This is quite analogous to the presence of two distinct DNA bands of nearly equal size found in CsCl density gradient runs on DNA isolated from *Paramecium bursaria* (Sagan, L., 1964, unpublished data). In this experiment, the larger DNA band corresponded to a base ratio of G+C = 29%, presumably, quite characteristic of the ciliates (Schildkraut, Mandel, Levisohn, Smith-Sonneborn & Marmur, 1962). The smaller band corresponded to 60% G+C, characteristic of chlorellae, and is presumably due to the presence of the photosynthetic zoochlorellar endosymbiont (Sueoka, 1961). The relative abundance of the chlorella band with respect to the nuclear, perhaps indicates the symbiosis is quite recent. Consistent with other data, it is possible that when our technology is more advanced, the quantitative relationship between the amounts of "main band" and "satellite band" DNA's on a CsCl gradient will directly indicate the relative amounts of metabolic responsibility relegated to the nuclear (main band) genome and, hence, to the age of the symbiosis in general. When we know the precise metabolic pathways and their genetic basis, we will be able to calculate directly the number of generations elapsed since each organism evolved from a common ancestor—from the number of mutational sites at which they differ.

Some searches will continue to be futile: for example, attempts to find eumitosis in all eukaryotes (if found, it will clearly be analogous, rather than homologous, to eumitosis in higher eukaryotes, cf. reports of sexuality in *Noctiluca*, a dinoflagellate); "missing links" in the origin of the ancestral phytoflagellate, such as organisms containing chloroplasts but no mitochondria; eumitotic organisms with bacterial flagella, or eumitotic fossils dating from anaerobic times.

The cytological basis of most "cytoplasmic inheritance" will probably be related either to mitochondria, (9+2) homologues, plastids, or other much less generally distributed cytoplasmic organelles (e.g. *Kappa* in *Paramecium aurelia*). As an example, the inheritance of the sr-500 gene in *Chlamydomonas* with the "plus" mating type (mt+) suggests that a correlated uniparental inheritance of chloroplast-specific DNA should be sought (Sager & Tsubo, 1961).

As in the past, future attempts to relate the various classes of algae directly to each other will be futile. For example, the ancestor of the relatively recent and isolated algae the *Bacillaracea* (diatoms), might be better sought among primitive metazoans than the chrysophytes or dinoflagellates. A simple flagellated organism with characteristic rhodophyte pigments probably never existed. A flagellated ancestor to the true molds and premitotic amoebae probably did not either.

The range of DNA base ratios in all plants and animals is quite limited compared to that in prokaryotes (Sueoka, 1961).

". . . The base of the Cambrian period ($0{\cdot}6 \times 10^9$ years ago) is marked in marine sediments around the world by the appearance of abundant animal life. . . . This sudden appearance of diverse animal stocks has been the most vexing riddle in paleontology" (Fischer, 1965).

The evolution of mitosis in ancestral heterotrophic amoeboflagellates, making possible mendelian genetic patterns and the tissue and organ level of biological organization, is presumably at the basis of both observations: the small variation in DNA base ratio in all plants and animals and the plethora of new forms of life marking the dawn of the fossil record.

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REFERENCES

ABELSON, P. (1963). *J. Wash. Acad. Sci.* **53**, 105.
BARGHOORN, E. S. & SCHOPF, J. WM. (1966). *Science, N.Y.* **150**, 758.
BELAR, K. (1915). *Arch. Protistenk.* **36**, 24.
BERNAL, J. D. (1957). The Problem of Stages in Biopoesis *in* "The Origin of Life on the Earth", Symposium of the International Union of Biochemistry, Moscow. New York: Macmillan.
BLOCK, K. (1965). *Science, N.Y.* **150**, 19.
BRACHET, J. (1957). "Biochemical Cytology". New York: Academic Press.
BUCHNER, P. (1930). "Tier and Pflanze in Symbiose". Berlin: Borntraeger.
BUCHNER, P. (1953). "Endosymbiose der Tier mit Pflanzlichen Microorganisms". Basil & Stuttgart: Birkhauser.

CALKINS, G. N. (1909). "Protozoology", p. 31. New York & Philadelphia: Lea & Febiger.
CLEVELAND, L. B. (1956). *J. Protozool.* **3,** 78.
CLEVELAND, L. B. (1963). Function of Flagellate and Other Centrioles in Cell Reproduction *in* "The Cell in Mitosis" (L. Levine, ed.). New York: Academic Press.
CLOUD, P. E., JR. (1965). *Science, N.Y.* **148,** 27.
COPELAND, H. F. (1956). "The Classification of the Lower Organisms". Palo Alto, California: Pacific Books.
CRONQUIST, A. (1960). *Bot. Rev.* **26,** 425.
DARLINGTON, C. D. (1958). "The Evolution of Genetic Systems". New York: Basic Books, Inc.
DILLON, L. S. (1962). *Evolution,* **16,** 102.
DOFLEIN, F. & REICHENOW, E. (1929). "Lehrbuch der Protozoenkunde: eine Darstellung der Naturgeschicte der Protozoen mit besonderer Beruchsichtigung der parasitischen und pathogenen Formen". Jena: Fischer.
DOUGHERTY, E. & ALLEN, M. B. (1960). Is Pigmentation a Clue to Protistan Phylogeny? *in* "Comparative Biochemistry of Photoreaction Systems". New York: Academic Press.
DUBUY, H., MATTERN, C. F. T. & RILEY, F. (1964). *J. Cell Biol.* **23,** 26A.
ECHLIN, P. & MORRIS, I. (1965). *Biol. Rev.* **40,** 193.
EDELMAN, M., EPSTEIN, H. T. & SCHIFF, J. A. (1964). *Proc. natn. Acad. Sci. U.S.A.* **52,** 1214.
EDELMAN, M., SCHIFF, J. & EPSTEIN, H. T. (1965). *J. molec. Biol.* **11,** 769.
EISENSTADT, J. & BRAVERMAN, G. (1963). *Biochem. biophys. Acta,* **26,** 319.
FEWSON, C. A., AL-HAFIDH, M. & GIBBS, M. (1962). *Pl. Physiol.* **37,** 402.
FISCHER, A. G. (1965). *Proc. natn. Acad. Sci. U.S.A.* **53,** 1205.
FRITSCH, F. E. (1935). "The Structure and Reproduction of the Algae", Vol. 1. London & New York: Cambridge University Press.
GABRIEL, M. (1960). *Am. Nat.* **94,** 257.
GIBBONS, I. R. (1963). *Proc. natn. Acad. Sci. U.S.A.* **50,** 1002.
GIBOR, A. & GRANICK, S. (1964). *Science, N.Y.* **145,** 890.
GOLDSCHMIDT, R. (1907). *Archiv Protistenk.* **8,** 84.
GOLDSCHMIDT, R. & POPOFF, M. (1907). *Archiv Protistenk.* **8,** 321.
GRANICK, S. (1962). The Chloroplasts: Inheritance, Structure and Function *in* "The Cell", Vol. II (J. Brachet & A. Mirsky, eds.). New York: Academic Press.
HALDANE, J. B. S. (1954). The Origins of Life *in* "New Biology, No. 16" (M. L. Johnson, M. Abercrombie & G. E. Fogg, eds.). London: Penguin Books.
HARTMANN, M. (1921). *Archiv Protistenk.* **43,** 223.
HOFFMAN, E. J. (1965). *J. Cell Biol.* **25,** 217.
JINKS, J. L. (1964). "Extrachromosomal Inheritance." Englewood Cliffs, N.J.: Prentice-Hall.
KARAKASHIAN, S. J. (1963). *Physiol. Zoöl.* **36,** 52.
LASCELLES, J. (1964). "Tetrapyrrole Biosynthesis and its Regulations", p. 17. New York: W. A. Benjamin.
LEDERBERG, J. (1952). *Physiol. Rev.* **32,** 403.
LEFF, J., MANDEL, M., EPSTEIN, H. T. & SCHIFF, J. A. (1963). *Biochem. biophys. Res. Comm.* **13,** 126.
LYMAN, H., EPSTEIN, H. T. & SCHIFF, J. A. (1961). *Biochim. biophys. Acta,* **50,** 301.
LYTTLETON, J. W. (1962). *Expl. Cell Res.* **26,** 312.
MARSH, H. V., GALMICHE, J. M. & GIBBS, M. (1964). *Rec. chem. Prog.* **25,** 260.
MERECHOWSKY, M. (1910) & MINCHIN, E. A. (1915). *In* "The Cell in Development and Heredity", by E. B. Wilson (1925). New York: Macmillan.
MINCHIN, E. A. (1912). "An Introduction to the Study of the Protozoa", p. 113. London: Edward Arnold.
PICKEN, L. (1962). "The Organization of Cells and Other Organisms", p. 259. Oxford, England: Clarendon Press.
PIRIE, N. M. (1959). Chemical Diversity and the Origin of Life *in* "The Origin of Life on the Earth", International Union of Biochem. New York & London: Pergamon Press.

PRESCOTT, D. M. (1964). Cellular Sites of RNA Synthesis *in* "Progress in Nucleic Acid Research and Molecular Biology" (J. N. Davidson & W. E. Cohn, eds.). New York: Academic Press.
RENAUD, F. H. & SWIFT, H. (1964). *J. Cell Biol.* **23,** 339.
RIS, H. & PLAUT, W. (1962). *J. Cell Biol.* **12,** 383.
RUTTEN, M. G. (1962). "The Geological Aspects of the Origin of Life on Earth." Amsterdam & New York: Elsevier Publishing Co.
SAGAN, C. (1961). *Radiat. Res.* **15,** 174.
SAGAN, C. (1965*a*). Ultraviolet Synthesis of Nucleoside Phosphates *in* "Origin of Prebiological Systems" (S. Fox, ed.). New York: Academic Press.
SAGAN, C. (1965*b*). Origins of the Atmospheres of the Earth and Planets *in* Section I of "International Dictionary of Geophysics" (S. K. Runcorn, ed.-in-chief). London: Pergamon Press.
SAGAN, L., BEN-SHAUL, Y., SCHIFF, J. A. & EPSTEIN, H. T. (1965). *J. Pl. Physiol.* **40,** 1257
SAGER, R. & TSUBO, Y. (1961). *Zeitschrift fur Vererbungslehre,* **92,** 439.
SCHILDKRAUT, C., MANDEL, M., LEVISOHN, S., SMITH-SONNEBORN, J. & MARMUR, J. (1962). *Nature, Lond.* **196,** 795.
SEAMAN, G. R. (1960). *Exp. Cell Res.* **21,** 292.
SIEGEL, R. W. (1960). *Expl. Cell Res.* **19,** 239.
SIEGEL, R. W. & KARAKASHIAN, S. J. (1959). *Anatomical Record,* **134,** 639.
SLEIGH, M. (1962). "Biology of Cilia and Flagella." Oxford: Pergamon Press.
SMILLIE, R. (1963). *Can. J. Bot.* **41,** 123.
STANIER, R., DOUDEROFF, M. & ADELBERG, E. (1963). "The Microbial World", 2nd Ed. Englewood Cliffs, N.J.: Prentice-Hall Inc.
SUEOKA, N. (1961). *J. molec. Biol.* **3,** 31.
TRAGER, W. (1964). The Cytoplasm of Protozoa *in* "The Cell", Vol. VI (J. Brachet & A. Mirsky, eds.). New York: Academic Press.
UREY, H. C. (1959). "Primitive Planetary Atmospheres and the Origin of Life" *in* "The Origin of Life on the Earth", International Union of Biochemistry. New York & London. Pergamon Press.
WALLIN, J. E. (1922). *Am. J. Anat.* IV, in Wilson (1925).
WALLIN, J. E. (1927). "Symbionticism and the Origin of Species". Baltimore: Williams & Wilkins.
WEINRICH, D. H. (1954). Sex in Protozoa: A Comparative Review *in* "Sex in Microorganisms" (D. H. Weinrich, chairman and ed.). Publication of the American Association for the Advancement of Science, Washington.
WENYON, C. M. (1926). "Protozoology: A Manual for Medical Men, Veterinarians and Zoologists." London: Bailliere, Tindall & Cox.
WILSON, E. B. (1925). "The Cell in Development and Heredity." New York: Macmillan.
YOW, F. W. (1961). *J. Protozool. Suppl.* **8,** 20.